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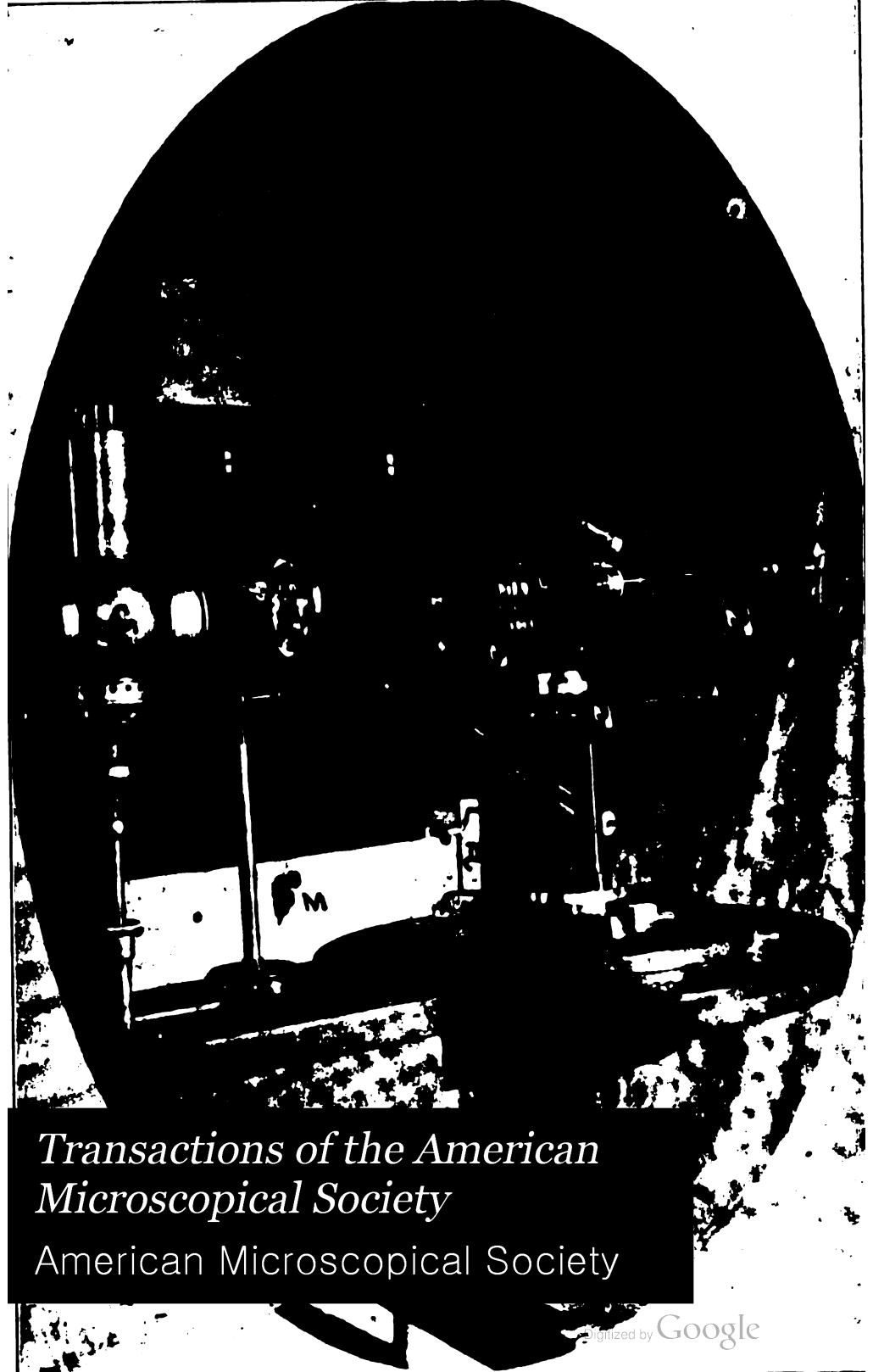

Transactions of the American Microscopical Society

American Microscopical Society

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TRANSACTIONS

...OF...

THE AMERICAN

MICROSCOPICAL SOCIETY

Organised 1878. Incorporated 1891.

EDITED BY THE SECRETARY.

NINETEENTH ANNUAL MEETING

HELD AT

CARNEGIE LIBRARY, PITTSBURG, PA.,

August 18, 19 and 20, 1896.

VOLUME XVIII.

BUFFALO, N. Y.
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9. T. J. BURRILL, PH. D., F. R. M. S., of Champaign, Ill., at Chautauqua, N. Y., 1886.
10. WM. A. ROGERS, A. M., F. R. M. S., of Waterville, Me., at Pittsburg, Pa., 1887.
11. D. S. KELLICOTT, PH. D., F. R. M. S., of Columbus, Ohio, at Columbus, Ohio, 1888.
12. WM. J LEWIS, M. D., F. R. M. S., of Hartford, Conn., at Buffalo, N. Y., 1889.
13. GEO. E. FELL, M. D., F. R. M. S., of Buffalo, N. Y., at Detroit, Mich., 1890.
14. FRANK L. JAMES, PH. D., M. D., F. R. M. S., of St. Louis, Mo., at Washington, D. C., 1891.
15. MARSHALL D. EWELL, M. D., F. R. M. S., of Chicago, Ill., at Rochester, N. Y., 1892.
16. JACOB D. COX, LL. D., F. R. M. S., of Cincinnati, Ohio, at Madison, Wis., 1893.
17. LESTER CURTIS, M. D., F. R. M. S., of Chicago, Ill., at Brooklyn, N. Y., 1894.
18. SIMON HENRY GAGE, B. S., of Ithaca, N. Y., at Ithaca, N. Y., 1895.
19. A. CLIFFORD MERCER, M. D., F. R. M. S., of Syracuse, N. Y., at Pittsburg, Pa., 1896.

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If the reader will be kind enough to make the following corrections, the writer's thought will be clearer. After making the corrections, this slip might be torn out of the TRANSACTIONS.

Page 330: *Second line from bottom, erase* (.

Page 331: *Seventh line from bottom, after* phase *insert* of.

Page 335: *Last line of second paragraph, change* telescoe *to* telescope.

Page 336: *Third line, change* become *to* became.
Fifth line, change affected *to* effected.
Ninth line from bottom, change that *to* as.

Page 340: *Sixteenth line, change* and *to* or the angle.

Page 341: *Seventh line of second paragraph, erase* this to be true :.
Tenth line of second paragraph, change aperture), *to* aperture).. *Erase* and that,. *Change* therefore, *to* Therefore. *After* power *insert* of *AE*.
Eleventh line of second paragraph, after great *insert* as that of the half aperture *BD*.

Page 346: *Last line, before d insert c.*

Page 353: *Thirteenth line, change* picture *to* pictures.
Fourteenth line, change differs *to* differ.
Third line from bottom, after of *insert* a.

Page 355: *Tenth line, change* coverglass *to* cover-glass.

Page 356: *Thirteenth line, change* 2 *to* 3.

Page 366: *Second line from bottom, change* diffracted *to* diffraction.

Page 380: *Twenty-first line, change* outward *to* onward.

Page 393: *Eighth line from bottom, after* lines *insert* three-fourths of. *Erase* and a half.

Page 13: *Third line from bottom, also bottom line, change* photo-micrographics *to* photomicrographs.

land, Chancellor of the Western University of Pennsylvania.

Mr. President, Members of the American Microscopical Society, Ladies and Gentlemen :

It is a very great pleasure to me on behalf of the local scientific societies and the citizens of this town to extend to

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[illegible] . . . . . Columbus, [illegible]

17. LESTER CURTIS, M. D., F. R. M. S., of Chicago, Ill., at Brooklyn, N. Y., 1894.

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PROCEEDINGS

OF

The American Microscopical Society.

MINUTES OF THE NINETEENTH ANNUAL MEETING

HELD AT

PITTSBURG, PA., AUGUST 18, 19, 20, 1896.

TUESDAY, August 18, 1896.

The members assembled in the Carnegie Library Building at 10 A. M. and were called to order by Mr. C. C. Mellor, of Pittsburg, Chairman of the Local Committee.

Members of the American Microscopical Society, Ladies and Gentlemen :

To my lot has fallen the pleasant and honorable privilege of beginning the exercises preliminary to the nineteenth annual meeting of the Society, and on this occasion, I will introduce to you two gentlemen, one of whom will make the address of welcome and the other the response, as it were touching the button, and these two gentlemen will do the rest. For the address the committee has been able to secure the citizen who is the best able to make it, one whose attainments are known the world over in his particular line and who is also at the head of the highest educational institution of this city. I have the honor to introduce to you Rev. W. J. Holland, Chancellor of the Western University of Pennsylvania.

Mr. President, Members of the American Microscopical Society, Ladies and Gentlemen :

It is a very great pleasure to me on behalf of the local scientific societies and the citizens of this town to extend to

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you on this occasion a most cordial welcome. Hospitality, as you all know, is an ancient grace and virtue, and I have heard it said by Pittsburgers that they excel in this virtue, and I, in fact, have heard others that have been in Pittsburg venture to intimate that the claim is just. There have been some historic interruptions to the hospitalities shown by Pittsburgers, notably when General Braddock kept the Indians on the other side of the Monongahela River during the French and Indian War. But away back in the days when Queen Aliquippa entertained George Washington, running down to the present time, there has been courtesy shown to the strangers, save and except when Captain William Trent, about 1772, acted rudely to the Indians who were rude to the Englishman, General Braddock. But these are all facts known to history, and the people of the present day may be relied upon to accord to you in their homes and in all the relations you may meet them a hospitality that will be personal. I welcome you as representatives of the learned of the nineteenth century. It is said of the most famous of the ancient Hebrew kings, accounted the wisest of his day, that "he spake of trees from the cedar which is in Lebanon to the hyssop which springeth from the wall; he spake also of beasts, creeping things (reptiles) and fishes." From this you will observe that King Solomon's knowledge was confined in botany to the phanerogams and that his knowledge of histology extended no further than to the lower vertebrates. He knew nothing of spores and bacteria ; all the wonders of mycetology and cryptogamic life were hidden from him. He knew nothing of the protozoa and the myriad forms of microscopic life with which you are familiar, representing the wonderful advancement of modern science achieved through the microscope. I welcome you as those who are wiser than Solomon, and who know more than the ancients, and trust from intercourse with you to add to the stores of knowledge. I welcome you as friends of humanity. People sometimes wonder why men should spend their time investigating mere minute organisms, spending months and hundreds of dollars.

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From the peculiarly economic standpoint, the investigator himself reaps very little return in fame or wealth, but the pathway is broadened and made plain to discoveries which enrich the world. You are representatives of those who with the microscope have carried our knowledge downward into the deep, while the astronomer gazing upward has made his way. Nature is most to be admired in things that are least known.

I welcome you in this ancient city, the city of industries, in which you will find anything that you wish to see, from a beautiful spectroscope, perfect in all its adjustments, to the grosser parts of such a mechanism as the man-of-war; where we make anything from a tack to a locomotive or an ocean steamer. I welcome you to a city in which we have something more than industries. Standing on the companion-way of a steamer a few days ago, I overheard a young lady say, "Where are those people from?" Her escort replied, "From Pittsburg." She said, "Where they have nothing but smoke and money." We have a great deal of smoke at times and there is a little money to be picked up in odd nooks and corners, I am told by some. But we have other things. This beautiful building, the gift of one of our citizens, the home of art and science; also the extensive park and conservatory. We have schools, colleges, hospitals and churches and learned societies and all those things that go to make the city a desirable place of residence in spite of its smoke. We have something better—a disposition to grow in knowledge and to make advancement in all lines open to us.

In the name of my fellow-citizens and the Iron City Microscopical Society I extend to you all a most hearty welcome.

Mr. C. C. Mellor—I now take pleasure in introducing to you the President of the American Microscopical Society, Dr. A. Clifford Mercer.

Dr. A. C. Mercer—President Holland, to you and the societies you represent and the citizens you represent, the society

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tenders its thanks for your words of welcome. The hospitality of Pittsburg is not an unknown quantity to this society. This society met here nine years ago and that meeting has gone on record as one of the best the society has ever had and we have no doubt this meeting will be at least a parallel. We know to some extent—probably only to a small extent, comparatively—something of the work done in Pittsburg to bring about this welcome, and for all those labors we are thankful. I have very little more to say. Personally, I belong to a calling which practises rather than preaches and it will be in accordance with that practice if we begin our work at once; therefore, I will declare the nineteenth annual meeting of this society open.

The names of a number of new members were then read, as recommended by the executive committee, and by resolution the secretary cast the ballot of the society for them and they were declared elected. They will be found in the list of members at the end of the volume.

Miss Edith J. Claypole, of Akron, Ohio, then read a paper on Notes on Comparative Histology.

Discussion:

Professor S. H. Gage—I suppose everyone who has used the microscope has studied this subject. It seems to me very suggestive that a subject that has been gone over so often has had a little new life put into it. Miss Claypole has considered it from the physiological instead of from the mechanical standpoint. There are at the present day two great schools of physiologists, those that believe physiology is refined mechanics, and those that believe it is something more than ordinary mechanics. Evidently she considers that physiology is something more than ordinary mechanics.

This paper has another beautiful feature about it that shows to the older ones as well as to the younger that there is not any subject exhausted yet. Every increase in knowledge makes an old subject a new one, and this subject has been made alive and interesting. It gives us more physio-

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logical insight, and so I feel sure that all of us will work with new zeal, and we hope next year Miss Claypole will bring with her some new tissues. I am sure she will be ahead of any of us.

Mrs. S. P. Gage—It has been very pleasing to notice in this study that the evolution of tissues is coming to be considered of equal interest with the evolution of the grosser structures.

Professor E. W. Claypole—We have the evolution of these tissues and these animals to consider. Unfortunately, from a geological standpoint, we can not get tissues, except in a few cases, to replace what these ancient creatures possessed in this respect. If we trust the embryologists, there must have been some change going on in the course of evolution of these animals on the earth, and it occurred to me that that is partly connected with the change that took place when land-life first began. As long as the reptiles were confined to the sea the animals possessed the advantage of breathing through their skins, but land-life deprived the animals of the power of breathing through the skin, and thus was increased burden of breathing through the lungs. The change took place somewhere in reptile life; that change was accompanied by the necessity for greatly increased oxidation of blood in the lungs.

We also have to consider such a question as this: Why should the camel alone among the mammalia possess these oval blood corpuscles? That is a question not yet answered by the paleontologists. The lamprey may be regarded as a highly specialised parasitic creature, because it sucks the blood of other creatures. The lampreys can be carried back to the Devonian era, and if they possessed blood discs almost spherical, then these must be perquisites of very ancient vertebrates. If the lamprey goes back to the Devonian age, it counts among the very early ones, and we have a nut to crack in this case. For that reason I think the possible outcome of this investigation may be of considerable interest to the

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members of this society who are interested in that part of the question.

Dr. V. A. Moore—I wish to endorse very heartily all that has been said, but I find, in looking at it from another standpoint, that the paper is very suggestive and opens up many subjects of profitable study. One of the most important of these is the diseases of animals. No tissue is more largely affected than the blood, and although much has been learned, still so little is known about its variations, changes and susceptibility to not only solids but those substances recognised as toxin and antitoxin. This paper opens up the field of the variability of structure of the blood in the same individual regarding atmosphere and temperature, food, and so on. I do not know of an exhaustive treatise on the blood of a single healthy animal, and it is on the healthy condition that pathologists base their knowledge of variations. It is important we should study the condition of the blood in a single specimen.

President—I think the discussion proves the paper is all that has been said of it.

The next paper was read by Professor S. H. Gage, on Courses in Histology and Methods of Conducting Them.

Discussion :

Dr. V. A. Moore—All of us who know something of actual work as a teacher will realise fully the profit which this practical talk which Professor Gage has given us will bring to those who are teaching histology. I was very glad of his suggestions in the beginning of giving the students some idea of the practical importance of this work. On the other hand, although I seem to change a little from my former statements, I believe in histology for histology's sake, and bacteriology for bacteriology's sake. Teach truth for truth's sake and not because it has some value or use one can turn into dollars. It is this seeking for truth that has led to the excellent paper of Miss Claypole's. We must look for truth to the men who have investigated the structure of tissues for their own sake. One thing not brought out by Professor

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Gage is the importance of the study of fresh tissues as well as those cut and hardened. Our knowledge of malaria and other disorders has come from the study of fresh tissues. Going hand in hand with what he said, this would be very helpful to a student.

Dr. V. A. Latham—I wish to thank Professor Gage very heartily for his suggestions. I hope that his forcible remarks may be made more so by being published, to encourage the medical colleges to assist the practical teachers. In my capacity as instructor in the medical college at Chicago, I find it very difficult to get room for my laboratory work. We are obliged in medical schools to take up and have in our classes pupils who have never seen a specimen under the microscope. We know Professor Gage's position is one of the foremost in the country. His classes of students are really the embryonic workers of the country today.

Professor E. W. Claypole—I wish he could get all the trustees of our institutions together and "lay it to them." If he could get such an audience as that, he might get a few converts. These remarks should be published and handed about, then some effect might be produced. As to what he said about the pathological value of histology, it is a difficult thing when students ask what is the use of studying histology. If you cannot talk in a way to make dollars and cents appear even in the distance, they shun the work. Not one in a hundred can tell the difference between abnormal and normal tissues. If I put an abnormal tissue under the microscope they know nothing about it. They can only send it to a specialist for examination.

Miss E. J. Claypole—I want to speak on one point especially. For two years I have been teaching in Wellesley College and one of my subjects has been histology. It seems to me one of the most important points in histology is to give students the tools to work with, afterwards the fresh tissues and methods of treatment, and if they have the opportunity they will be able to get at the structure of the tissues they have not examined from those they have examined. The

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knowing of specimens and treatment has been of so much use to me that I could not help speaking of it.

Dr. A. A. Young—I heartily agree with Professor Gage. He went no further than colleges—why not go to the public schools, where a child is taught how a drunken man looks without knowing how he got there?

President—Without calling for more discussion, I will ask Professor Gage to close it.

Professor S. H. Gage—I want to refer to just two things. When I advocated that we should show the students in the beginning what the good of the work is, I think that is correct, because unless one has a tremendous inclination for one subject over another, why should he select it unless he has that inclination? I want to bring about the study of histology for its own sake. A precious few only are capable of research. Those who have not this divine quality will get nothing out of it. But for the few who do have that, you do not have to tell them to study it for its own sake—they will do it after they get started. About the study of fresh tissue: I have said so often in the meetings—tried to impress it last year in my address—that the study of dead things has very little inspiration. It is like studying one thousand years from now the steam engine when we have changed our methods of transportation. How, under the sun, would one get any idea of what it was meant for, by taking it all apart a thousand years from now, supposing nothing remained to show what it was for? In histology, if we do not study the fresh specimens, we miss the pith of the subject. We may study them to get the structure afterwards, but study them alive and see what they can do. So, when life is in the tissue we are trying to understand, we can get some inkling of what it all means. Let us study the live histology and take the dead only as a necessity.

The Physician and His Microscope was the title of the next paper, read by Dr. A. A. Young, of Newark, New York.

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Discussion :

Professor S. H. Gage—That was not a false picture the author drew. I know a real good man and able physician, not a thousand miles from a certain university, who has just such a microscope ; very impressing to his patients. How much he knows about it is this : He bought some specimens and allowed me to look over them at one time. The one I examined was labeled stomach, but was evidently an intestine. On telling him that the specimen was not from the stomach, he said the man he bought it from knew more about it than I did and left the label on. Another brought me the result of an autopsy and wanted me to say what it was. I looked at it and told him it was no wonder the man died, because the pathological product was tea leaves. What I have always tried to do with the students who come to me is to tell them they cannot study pathology unless they study normal histology, and they cannot get it any more than German in fourteen easy lessons ; and when the students say they want to get into business as quickly as possible, I say, "Join the army of quacks ; they make $50 to a good physician's $1, and you will get rich."

Professor H. N. Conser—It is nearly always the case with physicians that they have had experience with the microscope, but the instruction is faulty. The matter of section cutting is naturally not very practicable for physicians ; they can leave that to those who have facilities for so doing. I think that is a fault in teaching.

The President appointed Professor Gage, Mr. Kuehne and Professor Kellicott on the auditing committee.

TUESDAY AFTERNOON.

At 3 o'clock the members assembled in the lecture room and were then conducted through the beautiful Carnegie Library building. Mr. Cunningham, the superintendent of the building, acted as guide and led the party through the museum, reading rooms, to the library proper, where the society was

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received by Mr. Anderson, the librarian. The alcoves, indexing room and study rooms were then inspected, and as the party was about to enter the auditorium an envelope was presented to each member, enclosing a card inscribed as follows: "Souvenir, nineteenth annual meeting of American Microscopical Society, at Carnegie Library, Pittsburg. Visit to Music Hall, August 18, 1896, at 3 o'clock P. M. Program: Andante in G, Calkin; La Cinquantaine, G. Marie; Reverie, Batiste; Overture, 'Zampa,' Herold; Hymn of the Nuns, Wely; Traumerei, Schumann; Offertoire, Wely. Organist, C. C. Mellor."

After thoroughly enjoying the organ recital, evidenced by the frequent and prolonged applause during and following Mr. Mellor's rendition, the society wended its way to the Phipps conservatory, in Schenley Park. Perhaps no conservatory in America can compare with the rich treasures of plant-life contained here, and the beautiful and rare species were greatly admired.

Tuesday evening, about 8 o'clock, the society assembled in the lecture room to listen to the annual address of the president, Dr. A. Clifford Mercer, of Syracuse, N. Y.

WEDNESDAY, August 19, 1896.

Meeting called to order at 10 o'clock.

President—Our first business is the nomination of a nominating committee to consist of five, and to report at the business meeting tomorrow. It will be satisfactory to the society and the executive committee if these business matters can be given as little time as possible, so that hereafter we can give almost all our time to papers and the more legitimate work of the society.

The members proceeded to nominate the following: Professor H. N. Conser, Professor D. S. Kellicott, Dr. V. A. Moore, Miss E. J. Claypole, Professor S. H. Gage.

President—It is moved and seconded that the secretary cast a ballot for these candidates as members of the nominating committee. Carried.

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Ballot has been cast in favor of these gentlemen and lady and this committee will report Thursday afternoon.

The names of a number of new members were then read as recommended by the executive committee, and by resolution the secretary cast the ballot of the society for them and they were declared elected. These names will be found in the list of new members at the end of the volume.

The paper of Miss Isabella M. Green, was presented by Professor S. H. Gage, on Ciliated Peritoneal Epithelium in Amphibia.

The next paper is on Photo-micrography, by use of ordinary objectives practically considered, with specimens of work by Thomas J. Bray, of Warren, O.

President—This eminently practical paper is open for discussion or questions.

Professor S. H. Gage—I would like to speak of one little device for the use of students. If we let these lines represent the camera and put an object on the floor there, we get strong shadows. We took the camera in its usual position and, in place of having an opaque object took a piece of glass, under which we put a white paper. That is very impracticable when we come to photograph large specimens, however.

President—We shall have in our proceedings a paper upon similar lines by another gentleman. Such papers will make our proceedings rich in this sort of practical instruction. None of us who has done any photo-micrography has listened without gaining some practical points out of the paper. And I think we have to thank the gentlemen who are not so much interested in tissues and the like, but give us the results of such work. Men who are busy in histological laboratories have little time to do such work. You will notice on the back of the photographs some notes. If everybody in presenting photo-micrographics for any purpose would give us similar notes it would be of immense value ; such notes ought to go with all photo-micrographics.

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In regard to the yellow screen, not only has it the value mentioned, but undoubtedly a yellow screen makes an objective which alone would not do good photographic work, capable of doing such work by photographing with the same yellow light for which the objective is corrected for visional purposes.

Then the third point. I think a word ought to be said about doctoring and improving negatives. That is all well enough from Mr. Bray's standpoint, to make attractive pictures, but when using a negative for scientific illustration it should not be touched in any particular; many a point may be developed or excluded to suit the imagination or theories of a person who is writing a paper, just as in the drawings; but the cardinal rule is that the print should come from the untouched negative, and the first negative and not the enlarged one, because in the act of re-photographing you may introduce changes. If we are making photographs for exhibitions and details are not important, then this is beautiful; but if beauty is not the object, but some truth we are after, the negative should never be touched in any of these ways.

Mr. T. J. Bray—I did not mean to touch the object, but simply the surroundings and show the object up more sharply; the negatives have not been re-touched, but simply the background.

The vice-president, Mr. Pennock, then took the chair, while the president read the next paper, on Astronomical Photography with Photo-micrographical Apparatus.

Professor S. H. Gage—Dr. Mercer in his papers usually covers the ground so absolutely and brings in matters that a good many of us are not familiar with. Certainly to have a matter of this kind brought before our society with the practical demonstration we have here of its results, is one of the gratifications we have in our annual meetings. Things come up most of us never thought of, and it certainly never occurred to me to try this on the sun and moon; but if it is

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not any harder than it seemed to be, I think we will all be observers of eclipses after a while.

The next paper, Notes on Technique, by Dr. P. A. Fish, of Washington, was read by Professor Gage; also a paper by the same author, entitled Zoophily versus Homophily.

Dr. V. A. Moore—I intended to prepare a paper for this meeting on the importance of animal experimentation, but on account of stress of work was unable to do so. It seems necessary to bring a few facts before the society, coming as I have from the hot-bed of this contest. It is difficult to tell just who started this bill originally, but it was presented by Senator Macmillan, of Michigan. It was brought by certain people belonging to the anti-vivisectionist society. They planned a bill entitled, A Bill for the Further Prevention of Cruelty to Animals in the District of Columbia. It is not necessary to read this, as it is quite an extensive document. While they had not claimed that they wished to prevent animal experiments, the bill if passed will prevent further investigation of the government in this line.

In the first bill presented to this committee, the law required that licenses should be issued by the District Commissioners to all people engaged in vivisection, and the District Commissioners should exercise judgment as to who were fitted to perform these experiments, putting into the hands of the District Commissioners the power to grant these licenses, which must be obtained for every experiment. These permits must be obtained five days before the performance of the experiments. You who are familiar with animal experimentation know that a delay of five days is sufficient to spoil all good that may possibly come from such work. You see it practically stops all work. In addition to that, these Commissioners were to appoint a committee of three from the Humane Society, so-called, who were to inspect laboratory work at any time they choose. You must open the doors and explain the investigation or experiment and stop all work and make an elaborate report to the District Commissioners whenever requested to do so. This bill

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came up before the District Commissioners and was opposed with as much argument as possible, but to no avail. The anti-vivisectionists procured a very oratorical lawyer and statements of experiments made in France thirty or forty years ago, and were successful. They did modify it somewhat, so that the committee should be appointed by the president and not by the District Commissioners. While it seemed unreasonable to think this bill could be reported, it was, and I have here the report on the same. It shows that the senators were fair in incorporating a good portion of the argument in opposition to it. It is on the calendar of the United States Senate and may pass that body immediately after convening of Congress in December. The people anxious for this bill have circulated petitions and have secured signatures of a great many men of prominence, bishops, clergymen and physicians. One of these has written Bishop Hurst a letter, saying that it was presented to him to prevent vivisection in schools, etc., in the District of Columbia, and we have found from consulting other people that they considered this bill of that character, and not of the sweeping nature it really is. It is one of the most outlandish, unreasonable combinations of proposed legislation that I have ever read, and its influence can be seen from the fact that these men and women who in the State of Massachusetts tried to get similar legislation through and then admitted that, if they can get this bill through Congress, they can use it in getting state legislation, so that really the time is coming when this country will be in the same deplorable condition that England has been for so many years. It is our opinion that vivisection in public schools should not exist. Laws in many states prohibit that. There is a law covering that in the District of Columbia. We have letters and a report from the commissioner there that vivisection is not practised in the schools, and it is not practised in the district schools anywhere, so far as I can learn. We do not want it, do not believe it humane and do not wish to use the life of an animal unless it is for a

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definite purpose. These remarks seem necessary to show just the condition and the only way in which this bill can be stopped. I think from reports that a large number of senators and representatives who have committed themselves to it, show that the average man is interested in this great question. The admission of such laws has cost England millions of dollars. For example, two diseases have cost England in money $400,000,000. The experiments necessary to find out the causes of these diseases and to find out how these diseases could be prevented would cost comparatively little. Think of the number of animals that have died from these diseases, to the amount in value of $400,000,000, and in this country we had about the same thing going on until this method was devised. All the diseases which have caused innumerable millions of lives are now rare occurrences, such as small-pox, cholera, and like diseases. It seems to me the number of animals that have been sacrificed is very few. On the other hand, I believe the animals used in these experiments suffer inconsiderably. It is only a question of premature death; but the question, it seems to me, does not rest on that. We have vivisection or animal experimentation for three legitimate purposes. The first is the demonstration of facts already known.

I believe it is necessary to experiment in medical schools. I believe it is necessary, and think every physician in this audience will agree with me, that every medical student should perform experiments on animals. Nobody, in my opinion, has a right to perform surgical operations on the human body until he is familiar with these experiments on the animal, which is always done with anesthetics.

Then there is the necessity for experimentation for the determination of facts, in which the knowledge to be gained has not any immediate utilitarian value, but may be of untold benefit ultimately. Some people think it is wrong to sacrifice life for this purpose.

Experiments made years ago determined facts that were looked upon as ultra scientific that are now indispensable

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to our knowledge. We are in possession of them because those men experimented for fact's sake and nothing else. We make these experiments for the ultimate purpose of determining facts which will save life and alleviate suffering.

There has been circulated recently, emanating from Philadelphia, a paper having a great deal of influence, saying that there is not any such thing as rabies, and that as a specific disease it does not exist. I do not know how many are familiar with the Pasteur method, but I venture to give a brief explanation of this. It was found by Pasteur and many others that if rabbits or other animals are inoculated with rabies they will die with certain paralytic symptoms. Then if we inoculate rabbits with healthy brains they remain well. Before this discovery by Pasteur, of people bitten by mad dogs about 48 per cent. died. This has been reduced by Pasteur's treatment to less than 1 per cent. This disease exists in this country to an enormous extent. We have had in the city of Washington fifteen cases of rabid dogs during the last ten months. One lady who was bitten by her dog died of rabies. The period of inoculation in a rabbit is less than in a human being. This lady was bitten in the face very badly, on the arm, shoulder and neck, by a dog, and the dog was brought to us and I inoculated two rabbits, and on the fifteenth day the physician who had charge came to me saying, "she is showing signs of rabies." We examined the rabbits and they appeared perfectly well. She died that night and the rabbits two days later. Again, a lady brought a dog for examination, wrapped up in a blanket on her arm, saying it had indigestion. I inoculated rabbits and they developed rabies and died on the twelfth day.

I wish to make a motion that the president appoint a committee from this society to draw up resolutions protesting against this or any other bill prohibiting animal experimentation.

President—Before taking my seat I would like to say a word about why this matter was brought out at this meeting. Back of this movement is something that is very strong. Back

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of this movement are money and influence, which are gaining sympathy all over this country, towards the end of the prevention of vivisection. It seems peculiarly strong, and it seems to be very thorough. While most of us who received notices have been in the habit of putting these circulars in the waste-basket and smiling, the power behind has been methodical and industrious, and has been wasting no time. It is pretty evident that its motive has been to take the citadel and then gain the country. It looks as if this power intended to capture the District of Columbia and then the country. We have an enemy and a strong enemy. We should share this interruption to our work with other scientific bodies; and as one of the bodies interested in this matter it would seem as though we ought to do something, at least to show how we stand on this subject, and for that reason the matter has been introduced.

President—I believe there is a motion before the society now to appoint a committee to draw up resolutions in this matter. How many shall be on this committee, Dr. Moore?

Dr. V. A. Moore—I would suggest either five or seven.

President—The question is open for discussion.

Dr. A. A. Young—It seems to me three will express the wishes of this society much better; I suggest a committee of three be appointed to report tomorrow.

Dr. V. A. Moore—I suggested five or seven. I am not sure that five is necessary. I simply thought that we have here a society of ladies and gentlemen, and that it would be well to have a committee comprising both.

President—Is there any discussion as to the matter proper?

Those in favor of the motion that the president appoint a committee to draw up resolutions to be presented tomorrow please rise; contrary the same. It is carried.

This matter has been somewhat under advisement, and it seems to the president and the executive committee that the view taken by Dr. Moore is correct and that the president

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will name as that committee Dr. V. A. Moore, the chairman, and the other members, Professor D. S. Kellicott, Mrs. S. P. Gage, Miss E. J. Claypole and Mr. H. von Schrenk. This committee will kindly prepare suitable resolutions and report to the society tomorrow.

The next paper, on The Acetylene Light as Applied to Photo-micrography, with illustrations, was read by W. H. Walmsley, of Chicago, Ill.

President—We will now listen to a paper by Dr. V. A. Latham, of Chicago, Ill. The title is, What is the Best Method of Teaching Micro-science in the Medical School?

Discussion:

Professor S. H. Gage—I do not think this ought to go without discussion. One thing impressed me very much, and that was the order of studies. It is certainly a very serious matter to have a person take up a subject he has no preparation for; it is a pure waste of time and that cannot be helped by the professor. We have in our own university excellent plans. We say no student shall take this course unless he has pre-requisites, for we say in the list of our courses, a man may take a special course in microscopy if he has had the preliminary course.

One point is not often recognised by teachers of science, and that has reference to drawing, which might be omitted. Most teachers of science will consider that heterodox, because there is a kind of belief in the minds of teachers of science that if a student only makes a drawing of his work then he has some definite knowledge. I think Dr. Latham is entirely right, that drawings amount to no more than a poor description made by the pupil. If the drawing is considered with great care by the teacher and the pupil can see that what he put down came from his head, and not from the object, then it is of value; and whether, in my courses, it is worth while for the pupil to draw everything I have my doubts and think it is not desirable. I think they ought to be made to draw in certain parts, but it ought to be of the same rigid kind

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and be criticised as severely as the examination paper, or the pupil will learn to draw not from the object before him but from his head. Some of the points in the paper are very pertinent to teachers of science.

Professor E. W. Claypole—I quite agree with what the writer says, also what Professor Gage has said. When I know, as I do, the kind of material that is dumped into our medical schools with the expectation that they in a short time will be turned out M. D.'s, I am surprised that the teachers can do anything with them at all. I have seen them go in and I know how they come out. I think a great deal of responsibility rests on those who teach in the medical schools, but in the common schools they ought to learn things that would be of use when they enter college or medical schools. Why should they not learn drawing at school? It is the proper place, and they have more time and ought to learn to use their pencils with some degree of skill and draw from the object before them. I remember a case where a student brought me a beautiful botanical drawing. I gave the author no credit for it and he wanted to know the reason. I said: "You drew the flower from one, the leaf from another and the stem from a third plant," and he knew it and sat down.

President—I think that many points could be discussed, but feel that we cannot discuss them for want of time.

I feel that the society appreciates what Dr. Walmsley has done with his acetylene gas and with his suggestions about the candle-power. It will be a pleasant thing for the society to be associated with it; and I can ask, in the name of the society, that Mr. Walmsley look into the matter and associate himself with some one he may select and perhaps another year bring before the Society a new candle-power standard.

WEDNESDAY AFTERNOON.

The society assembled at the Casino at half-past two o'clock and took the trolley cars, chartered by the local committee, for a trip to Homestead. Arriving at the Carnegie

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armor plate works the members were met by officers of the company and conducted through the various departments of the immense plant. The return to Pittsburg was made at five o'clock, and all were delighted and impressed with the scenes often described, but rarely witnessed.

WEDNESDAY EVENING.

At half-past seven the members of the society and invited guests from the city met in the lecture-room to inspect the photo-micrographs, to witness the demonstration of acetylene gas and to examine the microscopes, lantern slides and microscopical apparatus.

The list of contributors to the photo-micrographical exhibit included: Dr. R. L. Maddox, Southampton, England; Dr. Henri Van Heurck, Antwerp, Belgium; A. Nachet, Paris, France; Mr. Andrew Pringle, London, England; Mr. A. A. Carnell, Cornwall, England; James B. Shearer, Bay City, Mich.; Dr. A. Clifford Mercer, Syracuse, N. Y.; Thomas J. Bray, Warren, O.; George Rafter, Rochester, N. Y.; J. B. Shearer, Bay City, Mich., and others.

Mr. Walmsley demonstrated the excellent qualities of the acetylene gas by means of a lantern-slide exhibition, which was thoroughly enjoyed by all present. The microscopical exhibit was not as large as in former years, but was interesting and profitable. Too late were received prints from T. Comber, Esq., of Liverpool, England, and prints and lantern slides from M. T. Monpillard, Paris, France. M. Nachet sent, too late also, as a present to the society, a Daguerreotype made by Leon Foncault in 1844. He writes:

"I have great pleasure to offer to the American Microscopical Society a curious thing. It is a Daguerreotype made by Leon Foncault in 1844, and, what is most extraordinary, I have proof that he began his experiments on this subject three years before the date and signature engraved on the corner of the silver plate (say, only one year after the publication of the invention of Daguerre); so that the application of the process to microscopical photography followed imme-

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diately the discovery of Daguerre. This Daguerreotype with many others I have had the pleasure of owning for some months, were made for the engravings of a very remarkable work published in 1845 by Donné, the celebrated French histologist, and it was the first atlas of microscopical histology published after photographs. I hope this plate may have some interest for members at the meeting."

Ex-President Mercer is making an effort to retain the photo-micrographs, which arrived too late, for exhibition next year.

THURSDAY MORNING, August 20, 1896.

Meeting called to order at 10 o'clock.

The names of a number of new members were read, as recommended by the executive committee, and by resolution the secretary cast the ballot of the society for them and they were declared elected. They will be found in the list of members at the end of the volume.

President—The first paper is by Mrs. S. P. Gage, on The Development of the Brain in the Soft Shell Turtle.

Discussion :

Professor S. H. Gage—In connection with the brain of sharks, if I interpret aright, the curvature is more in the adult shark than in the earlier forms. In these the curvature is more like the embryo than in the reptiles and amphibians, which seem to me to have departed farther from the original type. This is seen more in the embryological forms than in the higher vertebrates and sharks.

Professor E. W. Claypole—We incline to the belief that modern sharks have departed from the ancestral form, because in the modern shark the whole front of the mouth and jaw have been turned down to the under surface of the body. The mouth is brought back away under the surface of the head, so that the shark can see nothing unless he turns over, because the mouth is on the under surface of the body, but in the ancient sharks the mouth was not in that position. In Japan the mouth is in front, in India the mouth is in front,

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but most all have changed to bring the mouth to the under side, and by the bending over of the brain and upper parts of the head this has been brought about, but I do not know to what extent.

Professor D. S. Kellicott—It has been my privilege to dissect the brains of the shark during the past year, and, of course, the mouth of the shark was underneath, but the axis of the brain was quite horizontal, as in the reptile.

Secretary—I wish to have the pleasure of complimenting Mrs. Gage. Her work last year was very much appreciated by the members and others. I am very glad to see she is continuing this work and hope she will continue to do so for many years to come. It seems to me that this saddle cleft she has spoken about has enlarged to the crura; and, if that is so, it seems to me that as the embryo develops, this space getting shorter, it may have something to do with the motor and sensory nerves going to the brain, because in the adult that portion of the brain is quite well developed, whereas in the embryo form it seems to be illy developed and the space large.

President—The next paper is by Professor E. W. Claypole, on The Teeth and Spines of some Fossil Fishes: Mazodus and Ctenacanthus.

Discussion:

Professor S. H. Gage—I used to read that the paleontologist, taking one of these teeth or spines, could reconstruct the animal perfectly, so that it would be practically as though the creature were before him. I was brought up on that belief, but when I saw a paleontologist set the head of one on the tail end of another and reconstruct an animal from half a dozen teeth, I came to feel that paleontology was about as uncertain as some of the modern studies and that paleontologists themselves are more amazed at the things which they are supposed to make than those which they actually can make.

Professor E. W. Claypole—We paleontologists are not the

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only sinners in that respect. At the same time, there have been some striking illustrations of what Professor Gage has said. But that the paleontologist can reconstruct a fish from its teeth is not true. If the tooth happens to be like that of some other fish, then we can construct that fish, but if the tooth differs from that of other fishes, we could not have reconstructed it.

The next paper is by Professor D. S. Kellicott, of Columbus, O., on The Rotifera of Sandusky Bay.

Discussion :

Professor S. H. Gage—Last year, when speaking of minute forms in water, I had no conception of the little things we are dependent upon. Taking life in the ocean, as whale, shark, cat-fish and so on, that are of use to us, we have very little conception of how they are possible. In a work like this on the plankton of these waters, we are getting some kind of notion how this life has a possibility of existence, and while in a certain way it is more striking to the general public to work on big things, I think finally it will be seen that the men who are working on the small things are getting at the real sources of knowledge and power. I suppose bacteriologists, and others, were looked upon as those who had no connection with this world, but were derided. Those who worked at bacteriology did some things that are of very great value as also those who labor in other fields, and we think it a great work for the community and all will ultimately understand this. It seems to me the descriptions given this morning of these creatures are abundant in themselves for intellectual enjoyment. The philosophic spirit is ground enough for the study of these, even if the economic questions were not involved. I study vertebrates almost altogether and not these lower forms, and it gives me great pleasure to hear about the other forms not familiar to me. The world's knowledge is so great that none can cover all of it, but if we can gain help of friends working in other fields than our own, it gives value to a society like this.

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President—There follow two papers regarding water supply. These two papers will be read this morning, the first one by Dr. M. A. Veeder on Public Water Supply for Small Towns, and the other by Dr. Wm. C. Krauss on The Requisites of a Pure Water Supply, leaving the discussion until afternoon.

THURSDAY AFTERNOON.

Meeting called to order at 3 o'clock.

Discussion of Drs. Veeder's and Krauss' papers :

Dr. Turnbull—The supply of water to cities and villages is one of the most important of sanitary matters. It is a question which is receiving a great deal of thought at the present time, and a great many of the states are taking individual action just at present. A few weeks ago I was in Albany, N. Y., and got laws of the State Board of Health, and found that outside of four or five cities, New York, Brooklyn, Poughkeepsie, Albany and Buffalo, the state board has entire control of this question. The law has not been so active in cities, which are allowed to go on with the present supply. Villages and towns have to comply with the requirements of the state board. The state board is really the supervisor and the state engineer is really at the head. No water works can be put in until the sanction of that board is obtained ; that really gives the state the control of the water supply, and likewise control of the sewage disposal and control of rivers and streams.

Dr. Veeder said this morning that most cases of typhoid fever occur in winter. I think they occur mostly in the fall. The germs increase during the hot months of summer, multiplying rapidly after a long period of incubation running from three to four or five weeks. Germs take longest in September and the disease reaches its height in the latter part of November. When severe weather comes there is a drop, and we find by the first or middle of January that the medium average for the year has been reached. In January it is below, and in March it is the lowest. As soon as warm weather comes on it increases and gets to the average in August, reaching its

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climax in October or November. I have here a chart which I compiled last year for Allegheny. I find that this chart compared with the chart of London for the past twenty-two years is almost identical. It is almost identical with that of Berlin. The fever in the city of New York reaches its climax in October or the latter part of September, showing that the disease is generated mostly in the warm months and reaches the climax before the cold weather starts.

Concerning filtration, I think that people generally believe that filtration is new. They say, what are the experiments in filtration, has it passed the experimental stage? And it is very hard to get them to believe that it has passed that experimental stage. It is perfectly practicable and has been for years. We have preached sanitary filtration here. Statistics for Allegheny and Pittsburg show that we probably lead the civilised world in the number of cases of typhoid. Allegheny leads Pittsburg. Last year in the city of Allegheny, with an estimated population of 120,000, 1,764 cases of typhoid fever were reported, and there were a great many unreported cases. Several hospitals did not report their cases; a great many cases acquired the fever here and were sent out of town unreported. Probably the total number would run over 1,800, or one case of typhoid to 69 of population. We have been trying for years to get a good water supply, and have got it finally so that the question is whether it is practicable. The plan consists of having a filter or crib four feet below the river bottom. The water runs through four feet of sand and gravel, flows into the crib, and from there is pumped into the reservoir. In other places below us, that has not been successful; they have almost as much typhoid as they had before. The only thing I can see where it would be of benefit is that it would take out a great many impurities. We get the water from about ten miles up the river. If the plan were carried out, the contamination would not be so great and we would get better water, but how much better, time alone will show, and I think, from all that I can hear, it is

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not successful, and measures have been taken for buying property and starting sanitary filtration. We are using the water of the Allegheny and Monongahela rivers. There has been an enormous death-rate for years. The State Board of Health has no authority in the matter; we have not got that far yet. I am sorry that I have got to tell you these things, you being visitors here, but I think these things should be told. We need a new water supply and I am hopeful it will come very soon.

Mr. W. H. Walmsley—I would like to say, in regard to Dr. Veeder's recommendation, that in Chicago we have an unlimited water supply in Lake Michigan. For some years the water had been taken from cribs, by means of tunnels. At that time there was very little pollution in the lake, but as the city grew we found it necessary to extend these tunnels. In 1893, a crib four miles out from shore was finished. We now find that when the wind blows from the south or southwest the water is carried toward and into the outer crib, and whenever it is found even in the slightest degree polluted, the warning cry comes, to "Boil the water." But, after all, I believe in cases where the contamination of water is even suspected, the best way is to boil the water and thus purify it from disease, especially typhoid fever.

Dr. A. M. Bleile—It is pretty well agreed that chemical examination of water has very little value except as to its hardness. I am almost prepared to believe, after a somewhat extended experience, that the bacteriological examination is unreliable, at least in this sense, and wish to urge this, in order to do away with the false sense of security, of the harmlessness of bacteria. That such water later breeds disease has been demonstrated more than once. The only thing is to watch the surroundings, and the engineer is better to do this than the bacteriologist. Unless examinations are made daily they have little practical value. After the germ is discovered, generally the mischief has been done. For instance, an outbreak of typhoid occurred in a hospital

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with which I was connected. The supply of water comes from reservoirs and during the day is used from wells. This water was examined by others and myself, and found to be very good. There was suddenly a case of typhoid fever. The patient was put into cottage 6. Two weeks after her admittance into cottage 6 a case broke out; a week later, another; in all, nine cases in that cottage. The cases were all confined to cottage 6. Now, these patients got the same food as the others. We investigated the dairy that furnished the milk; no typhoid about that, it being well kept. The ice used is artificial, alleged to be, but I doubt this. At any rate, there was nothing at all that we could find as giving origin to the contagion, and none of the 450 other patients, who had precisely the same supply as these, had developed a single case. I can not explain it. I firmly believe typhoid is water-born, or milk-born when water gets into the milk. We found nothing in water or milk. I believe some germs came into the utensils there at some time. Things are as nearly perfect as possible in such an institution. One explanation would be that typhoid fever is a contagious disease, but all evidence is against that, but I would judge from this case alone that it is contagious. But the point is, that at some time germs were introduced into the water, probably in the reservoir, had existed there for a little while and then died out. The town below now shows typhoid fever, although pure water exists there.

President—This will have to end the discussion, as far as others are concerned, but Dr. Veeder will please answer.

Dr. M. A. Veeder—The point in regard to typhoid fever in winter is not about the number of cases, but such cases as do appear—where they do occur they are more severe—that is the point I make. In reference to boiling water, I might say I mentioned that—I have been recommeding so doing for years. My plan is simple and successful—boil the water and have a large stone jar, fill it up and allow sediment to deposit in stone crock and in most localities there will be a large deposit of lime. Then the direct method would be to have

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in the house for that purpose, a large number of fruit jars or tight water bottles and put the water in them ; if not having ice put them on the cellar floor. People tasting this water will notice that it is clean and is much better than by using filters, which get foul and are not known how to be kept clean.

President—There has been one paper crowded out and only one, the author of which is here, Mr. Hermann von Schrenk. I think that he will be glad to give us some little account of his paper, occupying about five minutes, and then our program will be quite complete.

The secretary then read by title the remaining papers, being by members who have not been present :

Some Methods of Histological Technique, etc., by Dr. J. Melvin Lamb, of Washington, D. C.

Study of the Cellular Pathology of Carcinomas, by Dr. Clifford Walcott Kellogg, of New Haven, Conn.

Red Corpuscles in Legal Medicine, by Dr. Moses C. White, of New Haven, Conn.

Study of the Colorless Blood Corpuscles, etc., by Dr. M. L. Holbrook, of New York City.

Increasing Pollution of our Municipal Water Supply, by Dr. F. J. Thornbury, of Buffalo, N. Y.

Historical Diphtheria ; its Bacteriology, by Dr. C. F. Craig, of Danbury, Conn.

Historical Note on Photo-micrography, by Dr. A. Clifford Mercer, Syracuse, N. Y.

This completes the list, Mr. President.

President—We are now under head of ordinary business.

Secretary—I wish to say that all members who have read papers and have not handed them in will do so as soon as possible, as I wish to have the transactions out about the 1st of December, if possible, and surely before the holidays (applause), and if our president will set the example all will go well. If the transactions are delayed it will not be the fault of the secretary but of the members of the society.

President—Have you any other report ?

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Mr. M. Pflaum—There are two special committee reports. At the last meeting of the society a special committee of three was appointed to re-invest the Spencer fund.

To the American Microscopical Society :

The undersigned, a committee appointed at a meeting at Ithaca, to re-invest the Spencer-Tolles Fund, respectfully report :

After considering the question of receiving but ordinary savings bank interest at 4 per cent. and perhaps 14 per cent. per annum from a building and loan association, they decided unanimously in favor of the latter. A proposition was received from the Keystone State Building and Loan Association, of Pittsburg, showing that an investment of $400, if made before January 1, 1896, would in four and one-half years mature and yield the sum of $700, and thus would earn an average yearly interest of 16.66 per cent. The officers of the Association being personally known to your committee as men of honorable standing and of excellent business experience the committee unhesitatingly authorised the investment of the fund in said Association. The only care remaining to be exercised was to bring the fund up to $400, and to lose no interest in the transfer from Urbana, Ohio, to Pittsburg. The fund at the Ithaca meeting amounted to $372.27 ; the transfer was so made that full interest was received from the old investment up to January 1, 1896, bringing the sum up to $391.63, to which a contribution was made of $8.37, making the full $400, which began to earn interest from January 1, 1896.

We hope that results will prove that our judgment in this matter was not faulty. Respectfully submitted,

MAGNUS PFLAUM,
C. C. MELLOR,
C. G. MILNOR.

President—This report is before you ; what will you do with it ?

It was moved and seconded that the report be adopted and the committee discharged.

President—The fund will remain in the treasurer's hands after the discharge of the committee.

The motion was carried and the committee discharged.

Mr. M. Pflaum—The committee appointed at the last meeting reports as to the property of the society as follows :

To the American Microscopical Society :

The undersigned committee, appointed at the last meeting, to investigate the matter of the property and of a permanent home for the society respectfully reports :

1. That this society should adopt as a principle to gather no property of any kind, and to dispose of what it has on hand at the earliest opportunity.

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2. That, until such disposal, the property now on hand should necessarily remain stored at Pittsburg, the residence of the present custodian.

3. That as long as the society owns property it should, for the necessary convenience of the custodian, be under his direct control and be stored at his residence—a permanent storage place being, although for some reasons desirable, entirely impracticable.

Second, as to a permanent meeting place, this subject is one fraught with difficulty, and a very serious matter to determine.

A custom cannot be changed in a day. For nineteen years this society, wherever it met, has been guests and been treated as such. And there is no doubt the cities and the local membership therein, as well as the society enjoyed the meetings the more because of this relation of host and guests. Should now this custom be changed? Were the society to have a regular meeting place, then, the glamor of entertaining and of being entertained will vanish, and the society, perhaps, be the loser of many members on that account. The meetings will become dry and business-like and lack that spirit of "enjoying a vacation" which no doubt has aided in bringing many members to the meetings who would otherwise not have attended. Although the society contains many members who are animated solely by the love of science and do not care for the embellishments usually attending our meetings, yet it must not be forgotten that the society is still a missionary body, endeavoring to make converts and must use popular methods in its work.

The expenses of the meeting have always been borne by the local committee, aided by generous citizens. This will, of course, not be the case if the society settles as a permanent resident of a place.

From these points of view your committee cannot recommend a change of the present itinerating method of annual meetings.

The society has never as yet been without invitations from places where to meet, and we may safely trust the future to furnish the same hospitality as the past. But should it happen that no host offers himself, our executive committee may be depended upon to find one.

Respectfully submitted,

MAGNUS PFLAUM,
C. C. MELLOR,
C. G. MILNOR.

President—What will you do with the report of the committee? If it is moved to receive it, you can consider it later.

It was moved that the report of committee be received and that the spirit of the report be accepted and adopted. Seconded.

Professor D. S. Kellicott—I do not want to discuss this question with a spirit of opposition, but the first part of the

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report, the part which refers to custodian of property, I must say, does not commend itself to me. I can easily see how conditions may arise in which our property may be kept and administered without undue tax on the time and patience of some member of the society, and think I can see that our property with the volumes, if fragmentary, are worth holding for some time. Papers may be called for, and if the volumes are dispersed, are lost. I think it will be well to consider the question a little farther and give it more time, and see if it cannot be solved. It seems to me that would be better. It is not necessary that the treasurer should be the custodian; it is not necessary that the custodian should live in the same place with the treasurer. I feel, however, that there should be a permanent custodian, one who has a garret at his disposal and who could store the property. I really think it would be better than to dispose of our property.

The second part of the report, in regard to permanent place of meeting, I heartily agree with and will be glad to vote for. The country is too broad, too large, to have a single meeting place at the present, so I would really wish to have the question divided; that would be my desire, however the society may feel differently.

President—Will the mover allow this to be divided? If the second has no objection we will consider it divided. Now we will take up the first part of the question, the receiving of the report, and then we can take some action upon it afterward.

It was moved that the report be received. Carried.

A motion was made to adopt the second part of the report. Carried.

President—What will you do with the first part of the report?

Professor D. S. Kellicott—I will make the motion to lay the question on the table. Seconded.

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President—That will leave it open for discussion another year.

Professor D. S. Kellicott—We will then be one year older and wiser. We may have a good building and a cellar and a garret by that time. I may move to make Dr. Bleile the custodian another year.

The motion to lay on the table was carried.

President—There is a little business left over from last year. For instance, announcement was made at our last meeting that it would be desirable to make a change in the constitution, in Article II., so as to read:

Any person interested in microscopical science may become a member of this society upon written application and recommendation by two members and election by the executive committee.

Professor D. S. Kellicott—I move to adopt the amendment as now presented. Seconded.

Dr. A. A. Young—I would like to know the names beforehand of those who are getting in before they are acted upon by the executive committee.

President—I believe it is good policy at every step to get rid of business in our meetings and keep ourselves to papers. I think this amendment will aid us.

Dr. A. A. Young—If objection is had to a candidate, could not I have it in my power to make objection? By this means I could not.

Professor S. H. Gage—Our method was patterned after the American Association for the Advancement of Science. They elect by executive council, and there has never been any abuse. These men are all tested by two members of the society, who vouch for them under doubtful circumstances, and I rather think that Dr. Young overestimates the danger of getting the wrong people in. I do not think this is the kind of society the wrong people would get in. If it were "The 400" it might happen.

Mr. W. H. Walmsley—I know that this method was tried in several instances. I know that when names were read out

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it was the habit of no one to make objections; and in that way cranks were admitted. But when handled in committee cranks were sifted out.

President—We will have a standing two-thirds vote. Carried. The amendment is adopted.

President—Then there are some changes necessary in the by-laws. You will notice in your last copy of the constitution our old name still appears. That is simply a mistake and will be corrected without action; that is in section five. If the secretary will read By-law II. you will see necessity for another change.

Secretary reads:

The secretary shall edit and publish the papers accepted, with the necessary illustrations, in four numbers. The first number to be issued not later than October 1, after the meeting, and the remaining three numbers at intervals of not more than three months.

President—What will you do with this? I believe this was brought up a year ago. A resolution was passed by the society to the effect that the Proceedings be published in one volume instead of four, but no corresponding change was made in the By-laws. It remains for someone to move that the By-laws be changed to correspond.

It was moved and seconded that this change be made. Carried.

President—The third section of the By-laws needs some attention.

Secretary reads:

The number of copies of Proceedings of any meeting shall be decided at that meeting. Each author of papers accepted and published shall be entitled to twenty-five separates of his paper and as many more at the cost of publishing as he may request at the time that his manuscript is sent to the secretary.

Last year I had a great deal of trouble in getting twenty-five extra reprints for contributors, costing $75.00; and much trouble and worry to have reprints sent out, and in one instance the reprints never arrived at their destination. I propose, if the society is willing, to cut off extra reprints to members, at least for one or two years, to save expense.

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A motion was made and seconded that this section of the By-laws be remodeled or altered by leaving in simply the first sentence, the rest to be stricken out. Carried.

President—The secretary asks me to call attention to section IV. Reads:

Papers accepted for publication by the society shall not be published elsewhere until after they have appeared in the Proceedings of the society, except by consent of the executive committee.

Professor D. S. Kellicott—That was really rescinded years ago. Whether it got into print or not I cannot say, but my recollection is that the whole thing was rescinded while I was still secretary.

President—By oversight I was led to suppose that the election of officers could take place at any time, but it appears it should take place in the morning. But it seemed wiser to finish up all our papers and then have exclusively a business meeting. We will finish our general business and then proceed to the election of officers. Now is a suitable time to decide as to the number of copies of the Proceedings you will have for next year.

Secretary—I would rather the matter be left to the secretary and the treasurer. If there is not much saving let us get out the 500, but if there is let us get out only 400 copies.

President—I think it would be wise to leave this matter to these officers with discretion.

President—It is moved, by Professor Kellicott, and seconded that we publish 400 copies or more, according to the discretion of the secretary and treasurer. Carried.

President—I find on our records another suggestion of change in the Constitution, notice of which was given last year.

Secretary—It was moved last year by the secretary—a proposition submitted—that there be two conditions of membership for life, to add to our income. Reads:

A proposition has been submitted that there be two classes of members, or two conditions of membership for life, which would add to our income. The

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first is that persons or organisations desiring to obtain the Proceedings, by paying $50 would be considered as subscribing members, without any other rights of membership; while those paying $100 would be full life members, and entitled to take part in the proceedings of the society.

This is submitted as an amendment to the Constitution.

President—There is a notice before us of an amendment to the Constitution. What will you do with this motion? Will you take any action or not on the matter? Shall we have life memberships of two characters, one to cost $50, one $100, one to have Transactions and nothing more, and the other to have Transactions and privileges of meetings? If there is no motion we will consider that nothing is to be done with the question. There seems to be no motion. The matter will be dropped.

President—In regard to the printing of the Proceedings there seems to be another point about which we have a suggestion. It may be possible to make a reduction of expense by not printing the Constitution and By-laws separately. Last year the society passed a resolution to the opposite effect, saying that our secretary should print separately our Constitution and By-laws. If there is no objection the resolution will stand and the secretary will print separate copies.

Secretary—The price for printing the separate reprints was $15.00.

President—A motion to rescind the resolution of last year would simplify matters.

It was moved and seconded that the resolution of last year, authorising the secretary to print separately the Constitution and By-laws, be rescinded. Carried.

President—We have some committees to report, one on the vivisection matter, of which I believe Dr. Moore is chairman. Dr. Moore.

Dr. V. A. Moore—I have here a few copies of papers received this morning, in which this bill is analysed and the effect it will have on the animal industry of the country set forth.

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I furthermore desire to ask each member to write to the chairman of the District committee, or to the presiding officer of the Senate, protesting against the bill; also letters to the senators and representatives of his state. I think short letters, to the point, by different members of this society, will do a great deal of good in bringing about a just consideration of this bill. The committee desire to submit the following report:

WHEREAS, The American Microscopical Society, assembled in Pittsburg, Pa., August 18, 1896, for its nineteenth annual meeting, recognises the fact that the development of biological and medical sciences has resulted largely from experiments upon living animals; and

WHEREAS, Those engaged in experimenting upon living animals in scientific laboratories do so for the purpose of gaining the knowledge which may result in alleviating human suffering and improving the condition and value of domesticated animals; and

WHEREAS, No evidence has been presented by those who advocate restrictive legislation showing that abuses, such as cruel and unnecessary experiments, are practised in the District of Columbia; and

WHEREAS, Results of great scientific and practical importance have been obtained by experiments on the lower animals made in Government laboratories in the District of Columbia; therefore be it

Resolved, That the American Microscopical Society earnestly protests against the passage of Senate Bill No. 1552, entitled, A Bill for the Further Prevention of Cruelty to Animals in the District of Columbia, or any modification of this bill, or of any other bill restricting animal experimentation for scientific purposes.

Resolved, That copies of these resolutions, attested by the signatures of the officers of this society, and of its committee appointed to draft these resolutions, be sent to the chairman of the committee on the District of Columbia in the United States Senate and House of Representatives.

(Signed) A. CLIFFORD MERCER, *President*.
WM. C. KRAUSS, *Secretary*.
MAGNUS PFLAUM, *Treasurer*.
V. A. MOORE, Ithaca, N. Y., *Chairman*.
D. S. KELLICOTT, Columbus, O.
SUSANNA PHELPS GAGE, Ithaca, N. Y.
EDITH J. CLAYPOLE, Wellesley, Mass.
HERMAN VON SCHRENK, St. Louis, Mo.

President—The report of the committee is before you; what will you do with it?

It is moved and seconded that it be received and adopted

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by a rising vote. Is there any discussion or word to be said? Carried unanimously.

Professor D. S. Kellicott—It seems that those connected with public institutions would have much influence in this matter, and if we see that those institutions take a hand in it, it may have some value. I think it will be well to have the resolutions printed.

Dr. V. A. Moore—It is of great importance to bring this matter to the attention of different members of Congress. I have already seen the representative who represents my district in Congress, and he is heartily opposed to any such legislation. I think it would be wise to have some one see personally those who represent each member of this society in Washington—in addition to what may be done by institutions.

President—The executive committee has seen fit, under our Constitution, I believe, to recommend to this society the election of honorary members. As your president is somewhat responsible for this action by the executive committee, he would like to say a word or two about it. One candidate was our first president; he was also our second president. He has always been in harness up to very recently; but with advancing years his vacation becomes a matter of importance to him, and it is doubtful whether we shall see very much more of him in active meetings. His interest continues. You had a letter from him in which he excuses himself on those grounds. I refer to Dr. R. H. Ward, of Troy, N. Y.

The second candidate was our third president, Professor H. L. Smith, of Geneva, N. Y., and he was our president twice, as was Dr. Ward. He was in the harness until illness and death in his family brought life almost to an end with him. A letter from him is encouraging, indicating he is likely to be with us again before long.

The third is Dr. Maddox, of England. He has sent to this meeting photo-micrographs taken by himself many years ago.

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He is the Nestor of photo-micrographers and the discoverer of the present dry plates used in photography.

Professor S. H. Gage—I have the honor to move that General J. D. Cox, of Cincinnati, O., be also made an honorary member. I think the older members of the society recognise that we have in him a man who commands respect as a microscopist as well as statesman and historian and that we honor ourselves by electing him an honorary member. I will move that we add his name to the list of honorary members. Seconded.

Mr. M. Pflaum—I move that the secretary cast a ballot for the election of these members as honorary members of this society. Seconded. Carried unanimously.

President—Unless somebody has a reminder for me, it only remains for us to receive and act upon the report of the nominating committee. The nominations are :

For president, Professor E. W. Claypole, Akron, Ohio; for vice-presidents, Mr. C. C. Mellor, Pittsburg, Pa., Dr. A. M. Bleile, Columbus, O. ; for members of the executive committee, Dr. A. A. Young, Newark, N. Y., Mrs. Susanna Phelps Gage, Ithaca, N. Y., Dr. Wesley P. Manton, Detroit, Mich.

A motion was made that the report be received, committee discharged and secretary cast a ballot of the society for the election of these officers for the coming year. Carried.

President—The secretary has cast a favorable vote for these officers and they are our officers for the ensuing year. Applause.

Professor Gage—It is one of our great pleasures always at the close of the meetings to open our hearts a little, as the hearts of the people were opened to receive us when we came to the place of meeting, and it is with peculiar pleasure that I shall try to express, as well as I can, our feeling toward the local committee of Pittsburg. Every one who has had to do with the carrying on of the meetings realises after he has had this experience that there is a great deal to be done ; and

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every one connected with the society knows that a great deal of the success of the meetings depends on the way the local committee has smoothed the road, the doors and windows oiled and machinery moving so that everything is ready for us. I have been to a good many meetings of this society and always with great pleasure, with one or two exceptions, when we have had too much politics and am glad to say that we have not had any this year. Never have I been to one that has been better managed than this one. An extra good kind of oil has been put on doors and everything done to make our meeting successful as a scientific meeting. Everything possible has been done to give us that intellectual pleasure and enjoyment which is so great a part of a meeting of this kind. And, therefore, Mr. President, it is with peculiar pleasure that I move a vote of thanks to our local committee for making it possible for our meeting to be so successful.

President—It is moved and seconded that this society extend its thanks to the local committee for its great kindness, for its abundant work and the pleasure that the committee has given the society in many ways. Carried unanimously. The committee is therefore thanked with the full heart of this society for its work in the direction named.

What is the further pleasure of this meeting?

Miss E. J. Claypole—I am sure that everybody who has attended the meetings appreciates the fact that much depends on where they are held; and it gives me a great deal of pleasure to speak of the way in which we have had the opportunity of using this beautiful building and the hospitality shown to us by the Librarian, Mr. Anderson, and the Superintendent, Mr. Cunningham. I do not think that any student goes through a library or building like this without appreciation, and therefore, if it is in order, I would like to move a vote of thanks to these two gentlemen. Seconded. Carried.

Secretary—I move to extend a vote of thanks to the officials who were instrumental in permitting us to see the Carnegie Iron Works, on the afternoon of the second day, and

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also to Mr. Mellor a special vote of thanks for his interesting recital on the first day.

President—As a sort of general expression let this be a rising vote. Carried unanimously, and it is hearty I am sure.

Miss Claypole moved a word of appreciation to the superintendent of parks for flowers, which was seconded and carried.

Secretary—Last, but not least, we have to thank the press of this city for the able way in which they have treated us. Nearly every paper had one-half to a column or two columns about our meetings. I was told that it was unusual for the press to extend that kind of hospitality to such a small society, and I think it would be well to give a vote of thanks to the press for their kind treatment of the Microscopical Society. Seconded.

President—Carried, and our thanks will be extended in the proper way to the press. The secretary will attend to that.

President—Is there any further general business to bring before the society? If not, the time has come when my relation to you as president ends. I can assure you that, although it has caused me work, I have enjoyed every moment I have served you in this capacity. I have learned to feel more than ever before that I believe in the society. I see reasons for its existence, and I am pleased to work for it. I think that must be the feeling of every officer who undertakes the work connected with this society. As the work progresses the reasons for the existence of such a society become more and more evident. The letter, which perhaps you have read, the letter which I sent to all of you, has expressed some of these reasons, which I need not now repeat. I believe that there is a need for this society and that there is important work for it to do.

I thank you for your kind attention throughout the meetings. I feel grateful to my fellow-officers who have done so much of the work, and, of course, I feel particularly grateful to the local committee and especially to the energetic

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gentlemen who represented that local committee. I know they have suffered the pangs of discouragement, and I hope they have now grown out of that despondent condition and feel happier, because they must realise they have made this meeting a great success.

I again thank you ; and take the greatest kind of pleasure in asking Professor E. W. Claypole to relieve me of farther duty.

Professor E. W. Claypole—I have not much to say this afternoon ; my turn has not yet come. I thank you heartily for the quite unexpected honor conferred upon me, and I feel that it will be quite a responsibility, and I shall have to rely on your kind help in a great many ways in order to accomplish results next year. But, relying on you, we will promise to do our best at the next meeting and during the interval to further the interests of the society in any way it is possible. I own that I do not share the opinion that has been sometimes expressed, that this society has no true reason for its existence, for there is enough room, scope and work for a microscopical society between the Atlantic and Pacific, and the Canadian and the Mexican line. I do not know where a microscopical society could not find space in this sphere. There are subjects of which we know nothing. There are men and women who are able to investigate, whose attention has not yet been turned this way. We want to turn the thoughts and attention of students to fields where microscopical investigation lies. We want the help of practical students to help students who cannot afford to give time or money. If the Royal Microscopical Society of England can do its work and retain its position in that small country where work has been going on for ages before it began here, we see no reason why there should not be good, full and ample scope on this side, where the harvest is plenteous and laborers few. Railways will not give us as great advantages as we wish they would ; the consequence is that the workers are scant, but the bond that unites the members of this society should be sufficient, if they are really workers, to keep them in line

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and on the track of work. If I had time I could say some things in regard to methods that could be used to strengthen the society, but will conclude by saying that it will be my effort to serve you to the best of my ability during the year we are now entering.

Professor D. S. Kellicott—Our late president has now retired to the position of an ordinary member, and I suppose we may look upon him and speak to him and address him in the ordinary tones and manners. I suppose also, knowing him as I do, that he is a gentleman who would care to have our opinion of his work and opinion of him as a gentleman. I wish to move the high sense of regard in which we have held him as a presiding officer of this society during the past year.

I move that the society express its regard in a resolution. Seconded.

President—You have heard the motion put and seconded. Those in favor signify the same by rising. Carried.

Mr. M. Pflaum—I wish, on the part of the local committee, to extend an invitation to the society to attend a theater party this evening. We know the sublime is often closely connected with the ridiculous, and we thought you might enjoy a highly moral minstrel show. First, the place where this performance is to take place is in a theater. We will all start together from the Monongahela House ; after the theater, please stick to the local committee and we will lead you on in satisfactory ways.

Dr. A. C. Mercer—We have finished all our thanks and here this thing comes on—what are we going to do about it? I move we accept this very kind invitation from the local committee and thank them for another evidence of great kindness, consideration and forethought.

Seconded. Carried.

Dr. A. C. Mercer—Another matter ought to come up here. Some who have known of work going on through the year are aware that certain journals have done a great deal

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for the society, particularly two—the *Observer*, of which Miss Booth, one of our members, is the microscopical editor, and Mr. Smiley, of Washington, has given us a full page in the *Microscopical Journal* throughout the year. Letters have passed between Mr. Smiley and members of the executive committee and we have reason to feel that he has done a great deal for the society in getting membership and, therefore, I would move we extend our thanks to Miss Booth and others connected with the *Observer* and to Mr. Smiley for the help they have given us through the year. Seconded. Carried.

The society was then declared adjourned.

Thursday evening was pleasantly spent at a theater party, to the enjoyment of all who had the good fortune to remain, and thus ended, perhaps the most successful and certainly the most enjoyable meeting the association has ever held.

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TREASURER'S REPORT.

FOR YEAR ENDING AUGUST 12, 1896.

To Balance on hand at Ithaca meeting, Brooklyn Prize fund	$30.00	
" General fund	66.92	
		$96.92
" Membership dues, 1893, 1	2.00	
" " " 1894 8	16.00	
" " " 1895, 18	36.00	
" " " 1896, 224, less $1.04	446.96	
" " " 1897, 11½	23 00	
" " for meeting only	1.00	
		524.96
" Admission fees, 1895, 1	3.00	
" " " 1896, 26	78.00	
" " " 1897, 2	6.00	
		87.00
" Donation	5.00	
" Exchange	25	
		5.25
" Sale of Proceedings		90.93
" Contribution Brooklyn Prize fund	51.00	
" " International Bibliographical Bureau	25.00	
		76.00
" Spencer-Tolles fund		400.00
		$1,281.06

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By Postage	$35.75	
" Expressage	45.53	
" Stationery and printing	34.90	
" Sundries	31.95	
" Issuing Part 4, Vol. XVI.	123.43	
" " Vol. XVII.	478.50	
" Brooklyn prizes	81.00	
" International Bibliographical Bureau	25.00	
" Investment Spencer-Tolles fund	400 00	
	$1,256.06	
Balance on hand	25.00	$1,281 06

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SPENCER-TOLLES FUND.

To Amount reported at Ithaca meeting	$372.72
" Interest to January 1, 1896	19.61
" Contribution of Magnus Pflaum	8.37
	400.25
Less expenses	25
	400.00
To Interest to July 1, 1896	23.04
	423.04
Total increase during year	50.77

MAGNUS PFLAUM,
Treasurer.

We hereby certify that we have examined the foregoing accounts for the year 1895–1896, and find the same correct with proper vouchers for expenditures.

August 18, 1896.

F. W. KUEHNE,
S. H. GAGE,
D. S. KELLICOTT.

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NOTES ON COMPARATIVE HISTOLOGY OF BLOOD AND MUSCLE.

EDITH J. CLAYPOLE, PH. B., M. S., WELLESLEY COLLEGE, WELLESLEY, MASS.

The difficulty of basing general histology on books and discussions of human histology must have presented itself to anyone who has attempted to do it. Since almost all the standard reference books on the subject are written from a medical point of view for medical students the gap between standard books and the materials used becomes quite wide. Even if any mammal other than man is made the object of study, there is trouble since many of the tissues of the cat and rabbit, for instance, vary widely from the same tissues in man, while if any of the lower forms are used still greater differences are present. Compound tissues, in particular, differ, many layers well recognised in human tissues being absent or poorly developed. Many of the elementary tissues also are markedly different in the various animal forms. These points led me to examine some of the tissues of a few of our common animals to find the differences present, and if possible some explanation of them.

The animals chosen were the rabbit, cat, pigeon, turtle, snake, frog (*Rana viridis*), *Amblystoma*, *Cryptobranchus Necturus*, and also, to a slight extent, Amia a Ganoid fish, and *Protopterus* a Dipnoan.

The results are necessarily limited and somewhat incomplete, only two tissues being examined thoroughly.

Blood and Striped Muscle.—Some others are partly worked out, but not fully enough for discussion.

Blood, a tissue in form, if not in function, has received a very large share of attention, greatly owing to its medical

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and legal importance. Interest has always been centered on the size and number of the red corpuscles, a structure, so far as known, peculiar to vertebrates.

The corpuscles of all animals fall into two natural groups, those with nuclei and those without; these almost agree, with a division on the basis of shape, oval and circular, with but one exception, on each side. All mammals, so far as known, possess normally non-nucleated, biconcave, circular red corpuscles, with the exception of the camel tribe, which has oval cells. All other vertebrates—birds, reptiles, amphibians and fishes—possess nucleated oval corpuscles, with also one exception; the lampreys (*cyclostomes*) have circular nucleated corpuscles.

I have either made or collected from various sources measurements of the red blood corpuscles of as many forms in these classes as possible, with the following results placed in the form of a table.

Drawings were made from fresh preparations of *Amia*, snake, pigeon, frog, *Cryptobranchus*, *Necturus*, lamprey and man. The drawing of *Amphiuma* was made from preserved material. (Plate I.)

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SIZES OF CORPUSCLES.

	Oval.		Authority	Circular.		
	L.	B.				
	μ	μ			μ	
FISHES:						
Teleost:				Man	7.5	E. J. C.
Carp	15	9	Brass.	Mammals . . .	6.5	
Teleosts in general	18.1	11.4	Frey.	Lamprey Eel . .	12.6	E. J. C.
Ganoid:				Cyclostomes .	11.3	
Sturgeon	13	10	Welcker.			
Amia . .	11.6	8.6	E. J. C.			
Elasmobranchii:						
Ray and shark .	28.5	. . .	Frey.			
	22.6	. . .				
Dipnoan:						
Lepidosiren . . .	41	29	Welcker.			
AMPHIBIANS:						
Scaly	18.2	15	Frey.			
Frog	23.2	16.5	E. J. C.			
Toad	24	16	E. J. C.			
Triton . . .	25	16	Brass.			
	37.5	. . .	Frey.			
Megalobatrachus . .	47	33	Brass.			
Cryptobranchus . .	48 7	29.2	E. J. C.			
Necturus	58 4	31.1	E. J C.			
Proteus	58	35	Brass.			
Siren	59	30	Vailians.			
Amphiuma	75	45	E. J. C.			
REPTILES:						
Turtle	15	6	Brass.			
Snake	15.7	12.9	E. J. C.			
Lizard	16	10	Brass.			
	13	10				
Alligator	20	7	E. J. C.			
BIRDS:						
Fowl	12	7	Brass.			
Pigeon	12	7	E. J. C.			
MAMMAL:						
Camel	8	4	. . . . .			

These figures are very significant and suggestive. Variation ranges from 6 μ. to 75 μ. Among the heterogeneous class "fishes" there is marked difference. *Cyclostomes* have circular cells, quite small, 12.6 μ. in diameter. In Teleosts and Ganoids (carp and *Amia*), two of the more specialised groups, the size is about equal, 15 μ. x 9 μ. and 11 μ. x 6 μ. The size increases in Elasmobranchs, represented by the ray and shark, it is 22.6—28.5, (short diameter not given). The

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most generalised fish, the Dipnoan or lung-fish, possesses still larger corpuscles, 41 μ. x 29 μ. Here in this series specialisation is accompanied by decrease in size. (Plate II., Figs. 10–14) Again, among the Amphibians, the Cecilians, limbless, tailless, scaly, worm-like animals, the corpuscles are small, 18.3 μ. x 15 μ., nearly circular. Frogs and toads, very specialised in many respects, have corpuscles 22 μ. x 16 μ., approximately. *Triton* gives cells 25 x 16 or 32.5 x — both sizes are given, but there is no basis to judge which figures are correct. *Megalobatrachus*, the great Japanese salamander, has corpuscles 47 μ. x 33 μ. *Cryptobranchus*, the American cousin of this foreigner, agrees in size of these cells, 48.7 μ. x 29.2 μ. These are two air-breathing, tailed Amphibians, far more generalised in gross structure than the frogs and toads. The three following ones, *Necturus*, *Proteus*, the blind cave form of Europe, and *Siren*, all very generalised forms, make a group together, their corpuscles measure 58.4 μ. x 31.1 μ., 58 μ. x 35 μ., 59 μ. x. 30 μ. Last in this list is amphiuma, an air-breathing form, but possessing the largest corpuscles known, 75 μ. x 45 μ. Thus again among the Amphibians there is a regular decrease in size accompanying an increase in specialisation. (Plate II., Figs. 15–20.) But few forms of reptiles can be discussed, and there is less variation in those examined. Turtle shows corpuscles 15 μ. x 6 μ.; snake, 15 μ. x 12.9 μ.; lizard, 15 μ. x 10 μ.; alligator, 20 x 7 (Plate II., Figs. 21, 22). All are much smaller than those of the Amphibians, agreeing more with the Teleosts and ganoids. In the birds there is a still further decrease in size, the fowl and pigeon agreeing in corpuscles, 12 μ. x 7 μ. Finally, among mammals the circular corpuscles vary from 2.5 μ. to 9 μ., averaging 6.5. The only other circular cells known are the nucleated corpuscles of the lamprey and hag fishes, which are larger, as shown in the table; they average 12.6 μ. in diameter.

In this whole series there is a gradual decrease in size from the generalised to the specialised forms, not only in the different members of the classes, but also in the different

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classes themselves. At each end of the table are specialised forms—not equally so, but both far from primitive—modern fishes and birds and mammals. Between them are less specialised forms, and among these are found some of the largest corpuscles known. None of them are of greater interest than the amphibia, a class acknowledged to contain most widely varying forms, some highly specialised and others exceedingly generalised. The worm-like, degenerate Cecilians have small, nearly circular corpuscles (Plate II., Fig. 15), and from this there is a steadily increasing series, culminating in the fairly gigantic cells of *Amphiuma*. Passing away from amphibians, on the other side, the size again decreases, and, finally, in the mammals are some of the smallest cells known. There is one point more of interest to notice in these cells. It is, perhaps, only an accidental coincidence, but the cells of all the presumably degenerate forms examined are approximately circular, if not completely so. The lamprey 12.6 μ. in diameter; *Cecilian*, 18 μ. x 15 μ.; snake, 16 μ. x 13 μ.

There is another striking change in this series besides decrease in size. The normal absence of the nucleus from mammalian red corpuscles and the presence of it in all other red corpuscles is well known. Can the loss of this part be explained, a part so essential to ordinary cells? Consideration of the function of the red cell will assist in answering this question. It is no longer a typical cell; its protoplasm is highly specialised for one purpose, to hold hemaglobin (this substance makes 90 per cent. of the corpuscle), which in turn serves to take up, during the circulation of the blood through the lungs or skin, the oxygen essential for maintaining life. In all reserve cells the nucleus is the organ of division; through its means two cells arise from one. In other cells it may assume some other function. The red blood corpuscle with its protoplasm saturated with hemaglobin has become highly specialised for one function, to carry oxygen; the more hemaglobin carried, the more efficient is the cell in its work. The original use of the nucleus is lost, the work of the cytoplasm prevents it from acquiring a

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secondary use, it becomes a rudimentary cell organ, decreases in size and, when specialisation is pushed to the extreme, disappears altogether. In the most highly developed animals, the mammals, in which activity and power are greatest and most constant, and the necessity for rapid supply of oxygen to the tissues most urgent, the red corpuscles are reduced to a mass of protoplasm saturated with hemaglobin, a voracious oxygen eater. The nucleus is gone, it only occupied precious space, which is far better filled with the oxygen carrier. What else could be expected than this loss when the oxygen carrier is at such a premium? For loss it is, despite those who maintain the presence of a nucleus in mammalian red blood corpuscles. The advantage of the increased possibility far outweighs any use this rudimentary organ may have had, hence its loss. This disappearance is gradual, large in amphibians, the nucleus is reduced to a mere line in birds (Plate I., Fig. 3) to vanish entirely in mammals normally.

The gradual decrease in size of the cells is very readily explained on the same principle. Chemical exchange takes place far more rapidly and completely in many small masses than in a few large ones. A consequent decrease in size attended by a great increase in number takes place, resulting in a very large number, 5,000,000 per cu. mm. of 7 μ. bodies (man), against 56,000 of 58 μ. bodies (*Necturus*). The hemaglobin is increased in amount by loss of nucleus and rendered far more accessible by decrease in size of these cells. These advantages gained show reason enough for this law of decrease in size and the gradual loss of the nucleus in the adult corpuscle of mammals. Perhaps a series of careful observations on the blood of mammals and birds might show connecting links between the non-nucleated and nucleated corpuscles.

Here is a general law of decrease in size attending specialisation established for one tissue. Is this a general law applicable to all tissues? Blood is a tissue of a very special nature, and another possessing at least many similar qualifications is *striped muscle*.

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MUSCLE.

The ultimate structure of striped, or, as it is often called, voluntary muscle, is in many points even yet under discussion, many complicated theories being suggested as possible explanations of the appearances found under the microscope. These, however, are not the points to be discussed, but rather some others usually only incidentally noted, yet from a comparative standpoint of significance.

It is well known that the nuclei of the striped muscle fiber of mammals lie just under the sarcolemma or limiting membrane of the muscle fiber. In the frog the nuclei, as shown in transection, are scattered through the contractile substance of the fiber. The constancy of these respective positions shows them not to be merely accidental, but characteristic of each animal. Other points also appear of greater or less importance: size and shape of fiber; number, shape and size of nuclei; arrangement of fibers in fascicles and the structure of the sarcous substance as apparent from longitudinal and transections.

The following animals have been used; in all cases possible animals of the same physiological value were chosen; they were treated subsequently by the same processes in all possible cases: lamprey, *Amia*, *Protopterus*, frog, *Amblystoma*, *Cryptobranchus*, *Necturus*, turtle, snake, pigeon and cat. The results are arranged in the following tabulated form:

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Animal.	Av'rage Size of Fiber.	Range in Size.	No. of Nuclei.	Location of Nuclei.	Size of Nuclei.	Cohnheim's areas.	Coarse or Fine in Section.	
Cyclostome :								
Lamprey	10 μ	6–12 μ	1–2	Inside usually,	Small	Present,	Medium	Being small nuclei came to edge more often.
Ganoid :								
Amia	18 9 μ	15–21	1–2	Inside	Medium	None .	Fine	From esophagus.
Dipnoan :								
Protopterus	35 μ	21–45	1–2	Edge	Large	Present,	Fine.	
Amphibians :								
Frog	45	30–54	2–5	Inside	Small	Present,	Fine.	
Amblystoma	42.3	*30–57	2–3	Inside	Fairly large . .	Absent,	Coarse	Has dotted coarse effect by having few fibrils in a mass.
Cryptobranchus . .	78.6	6 –105	2–3	Inside	Quite large . . .	Absent,	Coarse, homogeneous	
Necturus	88.5	60–131	2–3	Inside	Very large . . .	Absent,	Coarse, homogeneous	Nuclei 45x9 μ.
Turtle	54	36 66	2–4	Inside	Medium	Absent,	Coarse.	
Snake	97.8	75–155	3–5	Inside	Small and oval .	Present,	Coarse	Dotted like Amblystoma.
	89.7	60–144	25–35	Inside	Small and round,	Present,	Very fine	Very fine and dense.
Pigeon	20.7	15–27	2–3	Edge	Small	Present, Absent,	Fine . . . . . Coarse.	Fine and coarse fibers.
Mammal	21.1	15–30	1–2	Edge	Small	Present,	Fine.	

* One fiber 87 μ.

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These facts do not run quite as smoothly as in the first case.

The line between the muscle fibers having nuclei imbedded in the sarcous substance and those having them at the edge, is drawn between the cold and warm-blooded animals—fishes, amphibians and reptiles—against birds and mammals, with one exception, the Dipnoan (*Protopterus*), in which the nuclei are at the edge as in birds and mammals; they are much larger, however, than in the latter fibers. The number of nuclei visible from a transection is, on the whole, the same, averaging 2-3, again with one exception, to be discussed later.

The terms coarse and fine have been used to describe the appearance of these fibers in transection. The explanation of this appearance is probably that the fibrils forming the fibers vary in size in different animals. A transection of a fiber involves transections of its constituent fibrils. If these are small the fiber looks fine-grained (Plate III., Figs. 29, 32; Plate V., Fig. 43); if large, a coarse effect results (Plate III., Fig. 30; Plate IV., Figs. 36, 38, 39). In longi-sections there is also a noticeable difference. In longitudinal view of ordinary striped mammalian muscle the cross-stripes are usually far more prominent than any others. In some of the more generalised forms the cross-stripes become merged into very much emphasised longitudinal striations (Plate III., Fig. 33; Plate IV., Figs. 35, 37). This difference can be explained by the varying size of the fibrils; if they are large their size gives the effect of longitudinal striations. In the largest these become more strongly marked than the characteristic cross-stripes.

One animal presents some very peculiar conditions. In the snake there are two kinds of fibers shown in transection; one is typical, coarse-grained, with 3-4 oval nuclei imbedded in the sarcous substance; the other is very dense, close, showing long cracks and lines across the surface, dividing it into irregular areas. The second marked difference between this fiber and the typical one is in the number

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of nuclei. Instead of 3-5 in each section there are as many as 25-35, an enormous increase. It appears from this view as if there were two distinct kinds of fibers. Examination of a longisection at once shows this to be untrue. These two apparently distinct structures belong to the same fiber; they pass abruptly into each other. The typical muscle is faintly cross-striped and rather clearly longitudinally striated. It has long oval nuclei scattered here and there through it. Suddenly this structure stops, the fiber becomes slightly larger in diameter, it loses all cross-stripes and nearly all longitudinal markings. The sarcous substance is dense and compact. Instead of a few oval nuclei there are a large number of small round ones scattered through it (Plate V., Fig. 41). So far as I know this is only found in snake muscle. Isolated material should be examined in order to fully investigate this peculiar condition; at present all that I can suggest is that it is some peculiar ending of the fibers. In the pigeon there is a slight difference also shown in cross-section between the muscle fibers; some appear coarse and others very dense, but in longisection there is no appreciable distinction to be made.

So here again is the same law, large fibrils in the generalised forms, small ones in the specialised. This is fairly consistent, the snake and dipnoan being the only exceptions.

The presence or absence of the so-called Cohnheim areas is also significant. These are divisions of the fibers (visible in transection) by irregular lines passing over the surface, probably caused by the massing of the fibrils into bundles. In specialised forms (lamprey, frog, bird, cat, Figs. 26, 27, 32, 42, 43) these areas are present, in generalised forms they are absent (Figs. 30, 34, 36, 38). The Dipnoan is a noteworthy exception to this rule, as the position of the nuclei also shows.

The question of size must necessarily be approached with caution. In form, muscle fibers are not so independent of physiological changes as are red blood corpuscles. A fully developed active muscle fiber is well known to be larger than

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an ill-developed one. Hence differences are to be expected between animals with good and those with but slightly developed muscular systems. This point must be borne in mind; also another, that all animals are not equally active at all times of the year, and that there may be a difference in size of fibers under the different physiological conditions to which they are subjected.

There is a great range in size among fibers examined, from 10 μ. to 97 μ. The smallest are those of lamprey, a relatively inactive animal, but it also has its other tissue elements small. The *Amia* and Dipnoan follow in this series in size.

The frog, strongly muscular when active, has fibers 45 μ. in diameter. *Amblystoma*, *Cryptobranchus*, *Necturus*, follow in gradually increasing sizes. Turtle is smaller, snake, an exception, larger, but bird and mammal are both small.

This series, in spite of variations, coincides fairly well with the series of blood corpuscles in all but one or two respects, which can be explained in part by the interference of other agents. In spite of increase in muscular power there is a gradual decrease in size from the generalised to specialised. No one would compare favorably the slow, sluggish *Cryptobranchus* or *Necturus* with birds or mammals in regard to muscular power, yet the former have far the largest fibers, which ought to indicate muscular activity, if size is the sole criterion. Very small size in *Cyclostomes* may be caused by specialisation and by lack of muscular activity. The one striking exception in size, the snake fibers, may perhaps be accounted for by its exceptional mode of locomotion. The difficulty of moving a limbless body over the ground is very great, and this demand for great force is satisfied by great increase in size of fibers. The peculiar second kind, possibly special endings, cannot at present be discussed. The other possible explanation in size lies in degeneration, or reversion rather, back to a primitive condition.

The change of position and decrease in size of the nuclei

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is perhaps easier to explain. In its physiological action the force is exerted in a lengthwise direction through each fiber, ultimately by contraction of fibers. The presence of nuclei, non-contractile masses, must prevent, to a considerable degree, the fibrils about them from producing the full effect of their contraction, since they must pull obliquely around the nuclei. Decrease in size of nuclei assists somewhat in preventing this waste by straightening the pull somewhat, but by pushing the interfering bodies to the wall, or, in other words, to the sarcolemma, completely obviates the difficulty; the fibrils can exert their full power and yet the nuclei remain to perform their function, whatever it may be. This is effected in the two highest classes—birds and mammals; in them muscular activity is at a premium in force and endurance. The decrease in size of the fibrils is also in favor of action; the same number of fibrils can be crowded into a smaller space and so more fibrils be present and a far more efficient nerve supply and blood supply. Owing to the small fibers the exchange between blood and tissue must be more rapid with small than large fibers.

While considering for a moment the results of these observations it may be objected to that blood is not a good tissue for basing an argument of this kind on. It is so intangible, always changing its chemical nature, always moving from place to place. But the very singleness of its aim renders it one of the best. Its function is the same for all animals, its movements practically the same; through this series the development of an essential mechanism can be traced, until it finally attains perfection in the highest forms of animal life, the mammals. Muscle is also a very specialised tissue, and is instructive in showing the mode of development along an essentially different line.

Briefly summing up we find the results, on the whole, harmonious; a general law can be deduced which applies to the main animal tissues. The more generalised the animal the larger the tissue elements; the more highly specialised the smaller are the elements. This is a very natural law;

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all growth and change in body depends on growth and change in cells forming it. It is but logical to expect such profound changes to show their influence on these important agents. Exceptions to it are found, of course, "no rule without an exception" says the old adage. Such should, however, be expected since the evolution of these elementary tissues is conditioned by the same laws as that of the animal as a whole. It is a commonly accepted fact that a form may be highly specialised in some lines and yet possess one or more very generalised structures. So an animal may, as a whole, be generalised in its tissues as Dipnoan (*Protoperus*), yet exhibit some one specialised tissue, for instance, its muscle marking it at once from the other tissues.

This same line of treatment can be equally well applied to any other tissues, simple or compound, but at present I have not sufficiently examined or studied them to discuss the results, though an interesting field of work is opened by this method of treating the elementary structures of animal bodies by the same methods as have long been used in comparative anatomy.

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PLATE I.

Red blood corpuscles (drawn from fresh material). Except figure 7.

Fig. 1. Amia—Ganoid.

Fig. 2. Snake.

Fig. 3. Pigeon.

Fig. 4. Frog.

Fig. 5. Cryptobranchus.

Fig 6. Necturus.

Fig. 7. Amphiuma.

Fig. 8. Lamprey (petromyzon).

Fig. 9. Human.

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PLATE I.

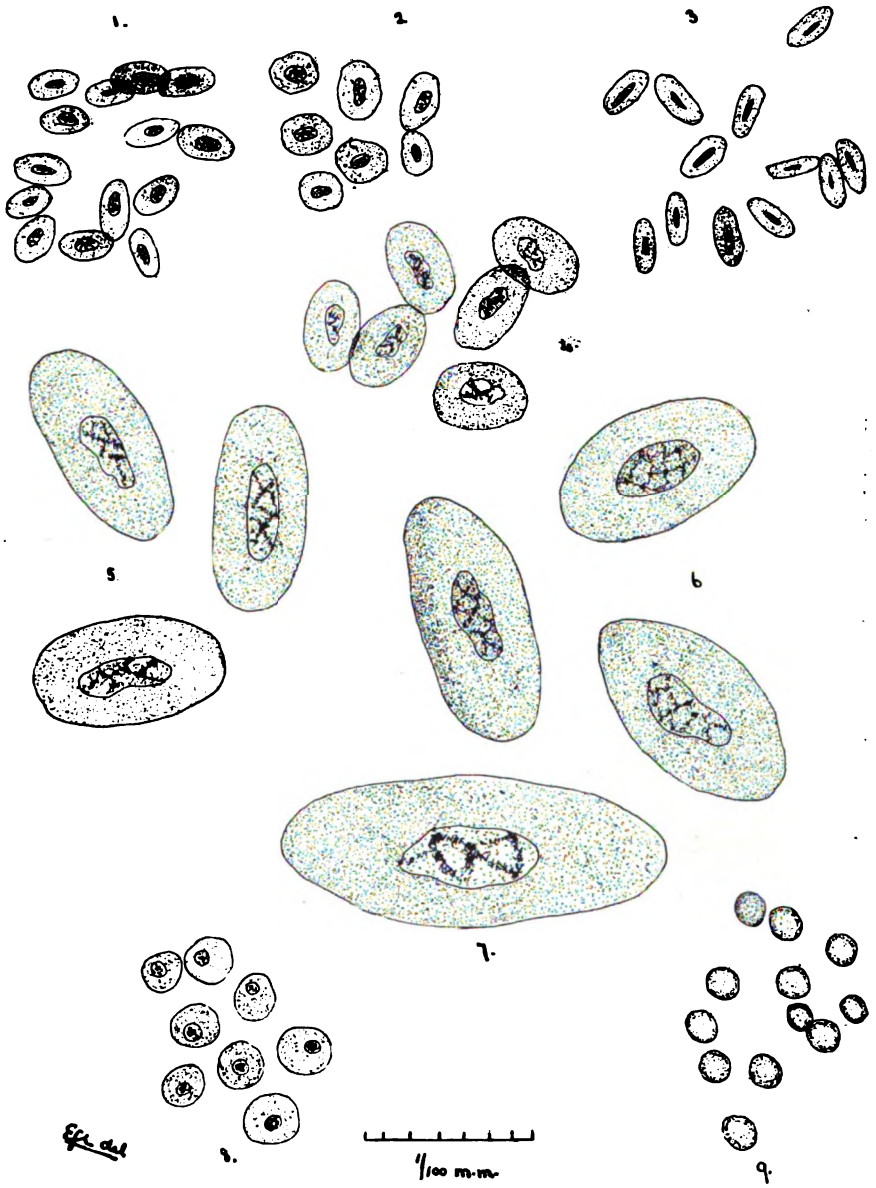

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PLATE II.

Diagrams of blood corpuscles.

Fig. 10. Lamprey (cyclostome).
Fig. 11. Carp (teleost).
Fig. 12. Amia (ganoid).
Fig. 13. Sturgeon (elasmobranch).
Fig. 14. Protopterus (dipnoan).
Fig. 15. Cecilian (amphibian).
Fig 16. Frog (amphibian).
Fig. 17. Salamander (amphibian).
Fig. 18. Cryptobranchus (amphibian).
Fig. 19. Necturus (amphibian).
Fig. 20. Amphiuma (amphibian).
Fig. 21. Turtle (reptile).
Fig. 22. Snake (reptile).
Fig. 23. Pigeon (bird).
Fig. 24. Camel (mammal).
Fig 25. Man (mammal).

Some of these are reconstructed from measurements.

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PLATE II.

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PLATE III.

MUSCLE.—Transections and longisections of striped muscle fibers.

Fig. 26. Lamprey. Body muscle.

Fig. 27. Amia. Esophagus.

Fig. 28. Protopterus. Longisection of body muscle.

Fig. 29. Protopterus. Transection.

Fig. 30. Amblystoma. Transection.

Fig. 31. Frog. Longisection (Sartorius).

Fig. 32. Frog. Transections.

Fig. 33. Amblystoma. Longisection.

Longitudinal striations shown in Amblystoma and are absent in Protopterus and frog.

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PLATE III.

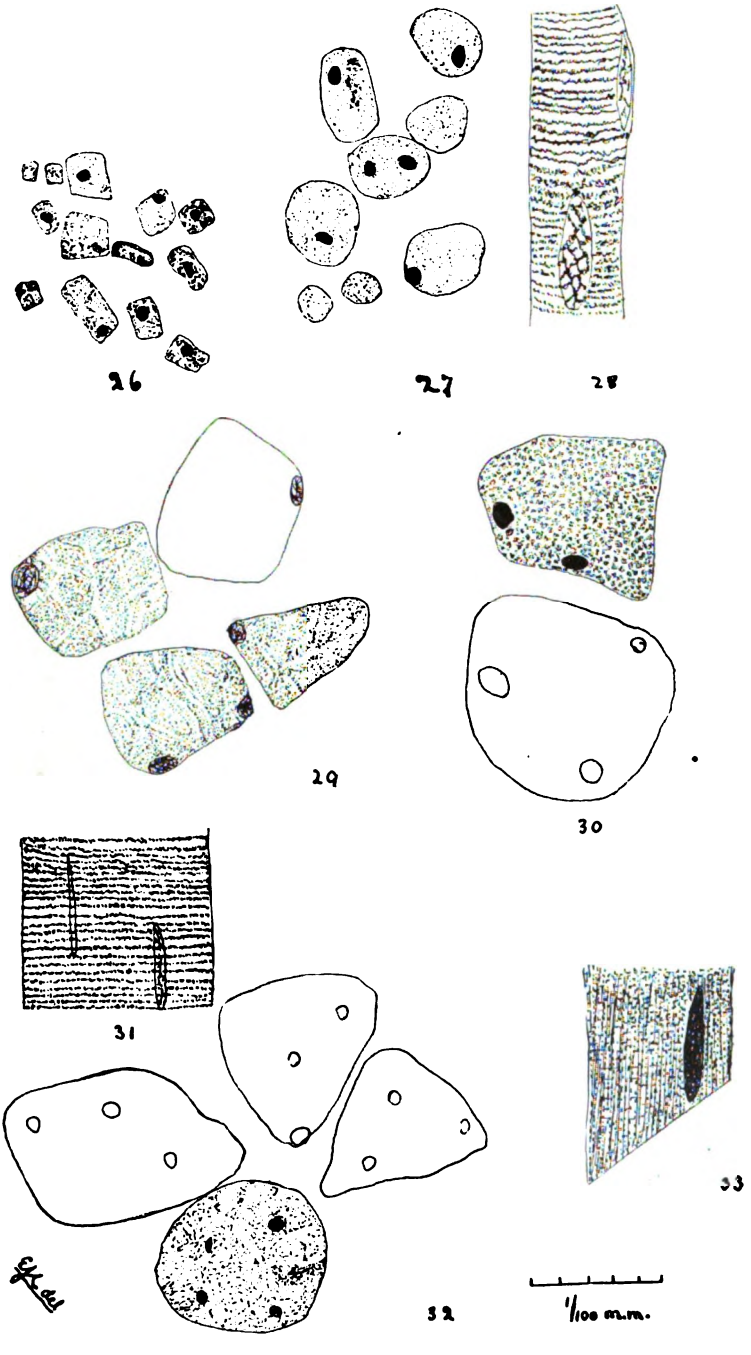

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PLATE IV.

MUSCLE—(*Continued*).

Fig. 34. Cryptobranchus. Transection of muscle in skin.
Fig. 35. Cryptobranchus. Longisection.
Fig. 36. Necturus. Transection of skin muscle.
Fig. 37. Necturus. Longisection of skin muscle.
Fig. 38. Turtle. Transection, from long retractor of head of animal.
Fig. 39. Snake. Transection. Coarse fiber.

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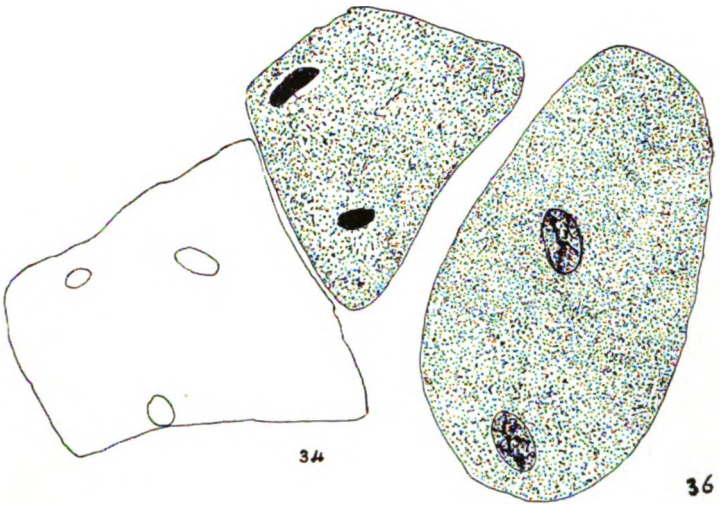
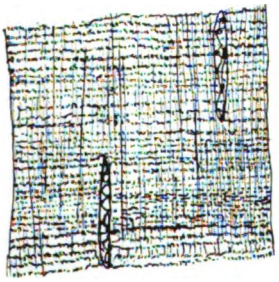
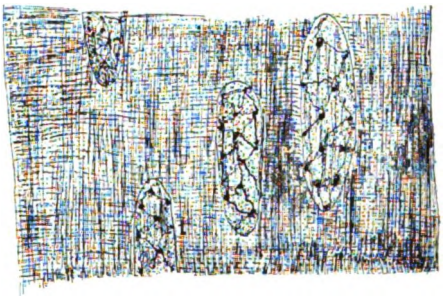
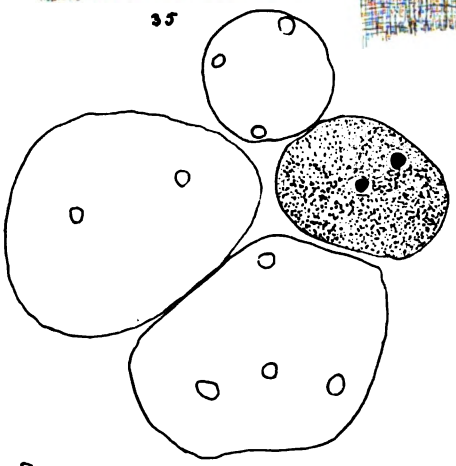
PLATE IV.









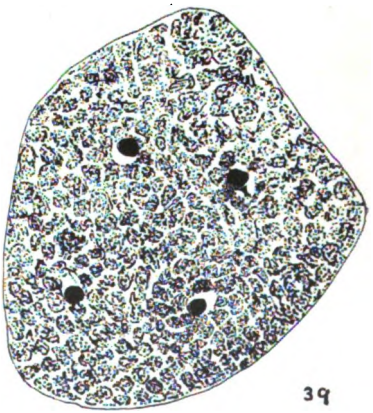


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PLATE V.

MUSCLE—(*Continued*).

Fig. 40. Snake. Transection of fine fiber, many nucleated (only partially filled in).

Fig. 41. Snake. Longisection showing the meeting of the two kinds of fibers and different forms of the two kinds of nuclei.

Fig. 42. Pigeon. Transection (from sartorius) showing two kinds of fiber in transection.

Fig. 43. Cat. Occipito-scapularis. Transection.

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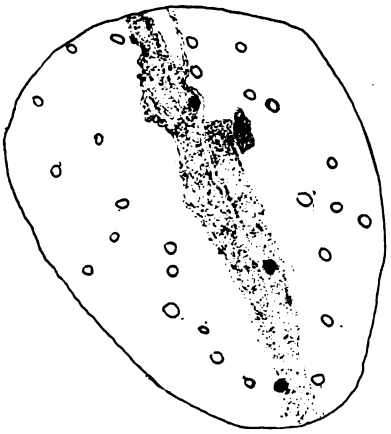
PLATE V.



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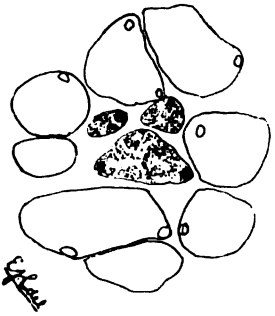

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THE PHYSICIAN AND HIS MICROSCOPE.

By A. A. YOUNG, M. D., Newark, N. Y.

One of the most expensive and one of the most useless pieces of office furniture that the ordinary physician possesses is his microscope. It usually occupies a most commanding and conspicuous place in the office and decorated with "fuss and feathers"; valueless as an educator, valuable for the macroscopical appearances of the microscope, for it is capable of producing wonder and awe to the office visitor and shekels to the pocket of the physician.

Nothing can be said against the microscope as an instrument, for its value resides in its intelligent use, and unless used intelligently it becomes worse than useless, distorting facts and fancies alike, from which the observer can form no concept, can draw no conclusion save an erroneous one. The physician has to deal with the organic world, with those material forms in which resides that peculiar, unresolvable and unknowable agent we call life, and without which matter becomes comparatively valueless.

The microscope in the department of medicine requires for its intelligent manipulation a familiarity with anatomy, pathology, bacteriology, and last, but not least, biology, which subject scarcely ever enters into a medical college curriculum. We, as physicians, must deal with material forms that are endowed with life, and of that relation which exists between the material form and life we must have some concept, though it be partial and inadequate, for on the relation of things material or immaterial is the development of human thought possible. The life force of the bacillus is doubtless as intricate as the life force of the human subject and may be similar if not identical with it; for what is the

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body in which the *ego* resides more than an aggregation of amebæ specialised, and each ameba possibly having an independent life and having reproductive properties of its own. It is with the minute mass of matter, not the molecule, that the microscopist has to deal; he sees its manner and method of growth and not the forces which produce the molecular arrangement of the ultimate particles.

It is not enough that the physician be able to observe and differentiate the various forms of the micrococcus, spirillum or bacillus: he must know as well the habitat, manner and method of growth of each variety. Without this knowledge the revelations of the microscope are no more intelligible than some Egyptian inscriptions. There is a philosophy of microscopy which is equally as valuable as the facts on which it is based, but a philosophy that can only be developed by accurate observation and classification of microscopical data. This work, it is evident, must be performed by the skilled microscopist and not by the novice, in which class the busy practitioner is usually found. In microscopical analysis no element relative to accuracy can with safety be omitted. It matters not though the microscopical accessories be thoroughly cleansed and sterilised, for the results would be equally untrustworthy if the material to be examined be placed in a receptacle, found perhaps in some old garret and half cleansed. Conclusions reached under such conditions must be erroneous. Do you ask who ever allows such procedures? Go to the home of the amateur or pseudo-microscopist, observe his methods and technique and you will have the answer. It is surprising how much we see, how much we assume and how little we know. A young physician asks an older one for the use of his microscope to examine a specimen of urine, assuring its owner that he is familiar with the instrument, having had instruction in college; permission granted, and slide prepared, and the observer exclaims, "The most beautiful specimen of a cast I have ever seen;" the owner of the instrument says, "That looks like vegetable matter and not a cast." "No," said the other, "that is a urinary cast; I have seen

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many of them." A microscopical examination of the container and its contents revealed a corncob for a cork; what the cast was you may readily infer.

A physician of several years' standing and the possessor of a good microscope at an autopsy of his announced that the patient's death was due to a disease of the kidneys, that she had been passing blood, pus, all forms of casts and other bad material with the urine. The autopsy, however, revealed ulceration with pus formation, degeneration and rupture of the gall-bladder, produced by impacted gall-stones, while the kidneys were practically normal, showing no structural degeneration. From whence, then, came the blood, pus, casts and débris, which was alleged to have been seen? These cases could have been none other than of mistaken identity; something was inferred that did not exist.

The conclusion is therefore reached, justly or otherwise, that the eye and understanding must be educated independently along certain lines before the manipulation of the microscope becomes satisfactory and trustworthy; objects must be seen and known relatively and in their entirety before being resolved into their component elements; the macroscopical appearance of an object must precede its microscopical appearance.

The physician must know in what menstruum and under what conditions the objects for which he is searching exists or are developed. Neither is it enough for him to know and recognise the various forms of bacilli; he must be able to classify them and know their manner and method of growth, what they produce by their growth and what influence they have upon humanity. This is the philosophy of microscopy as relates to medical science. The microscope therefore becomes to the physician valuable in the degree that he is able to classify and arrange its revelations so that they may be read as from an open book. This faculty means a familiarity with the instrument born of time,—time which the "country doctor" must give by piecemeal, if at all.

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I am no pessimist, although I see in a degree the passing of the microscope so far as it relates to the individual work of the ordinary medical practitioner. As already intimated, this passing is induced and sustained by unskilled and untrained eyes, which see much and individualise little.

The structure of microscopy, if it be enduring, must be built upon a comparatively errorless macroscopy. The rank and file still have to learn that the microscope only enables the investigator to continue his eyesight so as to observe the primary structure of an organised mass that would otherwise remain unknown and unknowable.

The first essential, then, for a physician microscopist is the proper use of his eyes, supplemented by a keen intellect; what he sees he must be able to describe accurately, thus differentiating the various forms and figures that appear in the visual field.

Neither is it enough for him to recognise an object in an isolated condition and know its form and construction: he must know as well what relation it sustains to other objects about it. This calls for the exercise of the comparative faculty, the second essential for the physician microscopist; indeed, these two elements may be called his eyes. With these faculties undeveloped, untrained, he may as well be a blind microscopist. What is true of normal vision is pre-eminently true of aided vision, which aid the microscope is, but it produces changes also in the relative conditions of objects, and of such changes the mind must take cognisance; it is an element too often overlooked. In short, the revelations of the microscope becomes the alphabet and the systematic arrangement of these revelations in the human mind forms its language, a language that requires study to comprehend; a language also that needs much further development and amplification. Physicians, as a rule, can be novices only in microscopical science, following where others lead; they stand at your feet, at the feet of the miscroscopists of the world, in the relation of pupil to teacher,

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asking for more light to illuminate the intricacies of human existence.

Give to them this light; save for them the microscope with all of its powers and possibilities which are vast; prevent it by your efforts from relapsing into a state of "innocuous desuetude."

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THE PERITONEAL EPITHELIUM OF SOME ITHACA AMPHIBIA.[1]

(*Necturus, Amblystoma, Desmognathus and Diemyctylus.*)

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This investigation was undertaken with a view to determining the character of the epithelium of the peritoneum of the tailed *Amphibia* of the Cayuga lake basin. The *Amphibia* selected for this work represent the orders *Proteida* and *Urodela*. Of the former, only one species, *Necturus maculatus*, is included, while three families of the *Urodela* are represented, viz., *Amblystomidæ*, by *Amblystoma punctatum; Desmognathidæ*, by *Desmognathus fusca ;* and *Pleurodelidæ* by *Diemyctylus viridescens*. One of the *Anoura*, *Rana virescens*, was used by way of comparison.

It was the intention to make a study of the various species at all ages and at different seasons. This, however, has been impossible, except in a few cases. No *Necturi* could be examined after spawning and *Amblystoma punctatum* was the only species taken in the fall. Immature specimens of *Necturus* and *Desmognathus* and larvæ of *Desmognathus* were studied. By combining all the species we have a series composed of (1) larvæ ; (2) immature ; (3) mature before spawning ; (4) mature soon after spawning ; (5) mature taken in August ; and (6) mature taken in the fall.

As regards the use of the term epithelium, authors differ. Ranvier, Klein, Kolossow and Piersol apply it only to the covering of the mucous membranes, using endothelium for the covering of the serous membranes. Paladino, Neumann,

1. This paper was presented to Cornell University for the degree of M. S., in June, 1896.

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Toureux, Hermann and Waldeyer discard the term endothelium, and call the covering of the serous as well as the mucous membranes epithelium. The term epithelium is used throughout this paper.

This investigation was carried on in the anatomical laboratory of Cornell University. I wish to express my appreciation of the facilities and material so generously placed at my disposal, and my gratitude to Dr. Wilder, Professor Gage and Instructor Hopkins, for their interest, encouragement and kindly criticism.

HISTORICAL.

For many years the serous membranes have been the subject of numerous investigations. Most of the work has been done upon mammals, but a number of articles have been written concerning the peritoneum of the *Amphibia*. The frog is the amphibian generally used, although Toureux ('74) worked upon the *Triton*, and Kolossow ('93) upon one salamander, two species of *Triton* and the *Axolotl*, in addition to the frog and toad.

Unfortunately their meagerness of data and details made it impossible to get the greatest good from the work of others. Often the statements were so vague that it was difficult to decide just what the author meant.

Among the first to work on the amphibian peritoneum was Mayer ('32 and '36). His results were not of great assistance, for although he spoke of finding cilia upon the peritoneum of the frog, the sex and age of the animal were not mentioned.

Valentine ('42 ?), in an article upon Ciliary Motion, enumerated the parts in a series of animals upon which cilia are found. Among them was mentioned the peritoneum of the tailed *Amphibians*, the *Triton* being given as an example. Cilia were found upon the serosa of the ovary.

Leydig ('57), in his Manual of Histology of Man and Animals, says that in the frog there are cilia upon some parts of the peritoneum, but not upon the mesentery, but does not

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state the sex of the animal examined. He also found cilia upon the mesoarium.

The next work was of a more specific character, being a paper upon The Presence of Ciliated Epithelium upon the Peritoneum of the Female Frog, by Thiry ('62). He found cilia upon the peritoneum lining, the ventral wall of the body cavity, and upon the membranes around the mouth of the oviduct, but none upon the serosa of the liver, and does not mention their occurrence upon any other part of the peritoneum. Thiry proved by experiment that the current produced by the cilia was in the direction of the mouth of the oviduct.

V. Recklinghausen ('62) was the first to use silver nitrate upon the epithelium of the serous membranes as a means of bringing out the cell boundaries.

Ordmansson ('63) spoke of finding stomates in the peritoneum of the frog and rabbit.

In 1863, v. Recklinghausen demonstrated by a number of experiments the presence of stomates in the peritoneum of the rabbit.

In the *Arbeiten aus der Physiologischen Anstalt zu Leipzig* for 1866 appeared four papers treating either of the epithelium of the serous membranes or of their relation to the lymphatic system. They were as follows:

1. Upon the Absorption and Excretion of the Pleural Wall, by Dybkowsky.

2. Upon the Centrum Tendineum of the Diaphragm, by C. Ludwig and F. Schweigger-Seidel.

3. The Treatment of the Animal Tissues with Silver Nitrate. Upon Epithelium as well as upon the Lymph Canaliculi of v. Recklinghausen as the Supposed Origins of the Lymphatic System, by F. Schweigger-Seidel

4. Upon the Peritoneal Cavity of the Frog and its Connection with the Lymphatic System, by F. Schweigger-Seidel and J. Dogiel.

In the first three of these papers is discussed the question of the communication of the lymphatic vessels with the peri-

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toneal and pleural cavities in mammals. Dybkowsky found small openings leading from the pleural cavity into the lymphatics. The others also found stomates.

The last paper mentioned above was especially helpful, both on account of the definite and careful statements made and the conclusions arrived at. The authors showed that (1) ciliated cells are present on the peritoneum of the adult female frog only; (2) the ciliated cells occur either singly or in groups among the non-ciliated cells; (3) ciliated cells are smaller and have more regular boundaries than the non-ciliated cells; (4) ciliated cells stain more deeply with silver nitrate than the non-ciliated cells; (5) the direction of the current is toward the mouth of the oviduct, and they therefore conclude that the cilia are for the purpose of aiding the ova to reach the opening of the oviduct; (6) the presence of stomates was demonstrated in the *septum cisternæ lymphaticæ magnæ*.

Afonassiew ('60) denied the presence of stomates.

Klein ('72) has written much concerning the serous membranes. In 1872, in a paper on Remak's Ciliated Vesicles and Corneus Filaments in the Female Frog, he described at length the ciliated vesicles found in the mesentery and mesogastrium of the female frog. He believed they are sinuses belonging to the lymphatic vessels and that, perhaps, they become ciliated only under pathological conditions. He mentions cilia as occurring between the stomates as well as on their borders.

In Klein's comprehensive work upon the lymphatic system he treated in Part I. ('73) of the serous membranes, and in Part II. ('75) of the lung. Klein demonstrated the presence of stomates leading from the pleural and peritoneal cavities into the lymphatics, and gave a full description of both the connective tissue and the epithelium of the serous membranes. He described the ciliated, germinating epithelium of the mesogastrium of the female frog as being present during the winter months, but he neglected to state whether this was the case in the male also and whether in

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the female the cells were germinating and ciliated during the summer.

Toureux ('74) made some investigations upon the *Triton* as well as the frog. He believed that the places which others took for stomates were merely depressions in the membrane.

The next year, 1875, two papers pertaining to the peritoneum of the frog appeared. The first one by Grunau ('75), treated of the ciliated epithelium upon the peritoneum and of the oviducts of the adult female frog. He found cilia upon the peritoneum lining the ventral and dorsal walls of the body cavity and upon the liver and membranes about the mouth of the oviduct and that the frequency of ciliated cells diminished as the distance from the mouth of the oviduct increased. Grunau stated that ciliated cells are smaller, but project more than the non-ciliated.

The second paper was by Neumann and Grunau ('75). They found cilia upon the peritoneum of the adult female frog and the *Triton*, the sex of the latter not being mentioned.

Toureux and Hermann ('76) considered the places which had been described by others as stomates as merely the centers of cell formation.

Nicolsky ('80) stated emphatically that "the cilia upon the epithelium of the serous membranes of the frog exist in all individuals without distinction of sex and age." He qualifies this statement, however, by saying that the cilia do not extend over so much space in the male and young female as in the adult female, and that "in the males the cilia are very short—one must search a long time for them."

Purser ('84) also went so far as to say that ciliated epithelium is found upon the peritoneum of the male frog, as well as the female, although not in such large quantities, and all this after having made the statement that the motion of the cilia is supposed to aid the ova in their passage to the mouth of the oviduct.

Some of the standard authors, such as Huxley, Quain

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and Piersol, mention the occurrence of stomates upon the peritoneum in both *Mammalia* and *Amphibia* and a number treat of ciliated epithelium upon the peritoneum of the frog, but in many cases no definite data as to age and sex are given.

Ecker ('89), in his extended work upon the Anatomy of the Frog, spoke of cilia being present upon the peritoneum, but did not state definitely whether this was the case in both sexes and all ages.

Kolossow ('93) worked upon two species of *Rana*, one of toad, two species of *Triton*, one of salamander and the *Axolotl* and found cilia only in the adult female of the *Triton*, frog and *Axolotl*.

PERITONEUM.

The peritoneum is the serous membrane lining the abdominal cavity. It is a closed sac, excepting in the female, where the opening of the oviducts break the continuity. In *Amphibia* the parietal peritoneum tightly adheres to the body wall, and the visceral completely invests all the viscera, with the exception of the kidneys, which are covered only on one side. The diagrams (Figs. 1–3) show the peritoneum as it appears in sections cut through the peritoneal cavity of *Necturus* at different levels.

The peritoneum is developed from the mesoderm. According to Minot ('92) the specialised layer of the mesoderm, called mesothelium, lines the body cavity and covers parts of the viscera. At first the cells of the mesothelium are cuboidal and cylindrical, but as development goes on they become reduced in thickness and finally reach the thin plate-like form of the epithelium. From the mesothelium is also derived a layer of mesenchyma, which finally becomes the connective tissue stratum of the peritoneum.

The peritoneum is a thin shining membrane, through which the different organs can be plainly seen. It is composed of (1) the connective tissue stratum and (2) a single layer of epithelial cells.

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The first consists principally of bundles of white connective tissue. In *Necturus* these form a thick network. The fibers are arranged in wavy rows, either parallel or crossing each other. (Fig. 4.) Numerous fine elastic fibers are present. They often branch, as is shown in Fig. 4. In the mesentery of the small intestine were found bundles of smooth muscle cells. Although not very numerous some were of quite good size. (Fig. 5.) A single cell is shown in Fig. 6. The nuclei are thicker and more rounded than the typical rod-shaped nuclei of smooth muscle cells. In the peritoneum lining the dorsal wall of the body cavity of *Necturus* are numerous pigment cells which branch and anastomose. (Fig. 7.) Pigment cells occur in different parts of the peritoneum in the various *Amphibia* studied. In *Diemyctylus*, the peritoneum covering the dorsal wall of the body cavity and the testes, is very densely pigmented. In all of the *Urodela* examined there was some pigment in the peritoneum, principally in the serosa of the testis, the dorsal wall of the body cavity and the mesentery of the intestine.

The epithelium of the peritoneum is a simple squamous epithelium. The cells are united by a thin layer of cell cement. For determining the character, shape and size of the epithelial cells and their relation to each other, three principal methods of investigation were employed : (1) The examination of fresh material in blood serum or normal salt solution. (2) The application of silver to the fresh material. (3 The preparation of sections. The first method is especially recommended by Klein in his work on serous membranes. It proved very advantageous in detecting the presence of cilia, as they are visible with a high power both in motion and at rest. The method of procedure is as follows : A bit of the peritoneum is spread out on a slide in a drop of blood serum or normal salt solution and covered with a coverglass, care being taken not to rub or tear the epithelium. With a 2 mm. oil immersion objective the cilia can be seen, both on the edge of the preparation in profile and also on the surface in face view. Normal salt solution proved as good a

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medium as blood serum. At first the cilia move so rapidly that they can scarcely be seen, but the speed gradually slackens until they only move a few times a second and finally stop altogether. In the fresh preparations the nuclei are visible but the cells are not demarcated.

OCCURRENCE OF CILIA.

The ripe ova are shed into the abdominal cavity and must pass cephalad to reach the mouth of the oviduct. The question of the presence of cilia upon the peritoneum of *Necturus* is a very interesting and important one, as up to the present time no one has published any statements concerning the passage of the ova from the ovary to the oviducts or the finding of ova in the oviducts. According to Marshall ('93), in the frog the ova are propelled partly by the muscular action of the body walls and partly by the motion of the cilia on the peritoneum. So far as I know, the first means has not been demonstrated, and it is hard to see how the muscular action of the body walls would tend to force the ova into the oviduct. If the sole purpose of the cilia is to propel the ova towards the mouth of the oviduct, then we should expect to find cilia in the adult female and at the period of ovulation only. It is possible that cilia may be developed in the female at maturity and then remain during the rest of the animal's existence, but their presence would not be looked for in the male and young female. Hence, it seems strange that Nicolsky and Purser described cilia upon the peritoneum of the male as well as the female, even if only in small quantities.

In both sexes of *Necturus* the peritoneum was thoroughly examined for cilia in the following parts: the hepatic ligament, the ventral wall of the body cavity, the dorsal wall of the body cavity, the membranes near the mouth of the oviduct, the mesopneumon, the mesogastrium, the serosa of the stomach, the serosa of the liver, the serosa of the small intestine, the mesentery of the small intestine, the mesentery

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of the large intestine, the spheno-hepatic ligament, the gastro-hepatic ligament, the gastro-splenic ligament, the serosa of the ovary, the mesoarium, the serosa of the testis, the mesorchium, the peritoneal fold supporting the oviduct, and the peritoneal fold supporting the vas deferens.

In *Necturus maculatus* cilia were found only in the adult female. The male *Necturi* were destitute of cilia upon the peritoneum (Fig. 8) and females, in which the ova were only one-third the size of those which would be laid in the spring, showed the same condition as the males. These immature females measured 28 and 29 cm., but in one female only 28 cm. in length, cilia were as long and as widely distributed over the peritoneum as in the large specimens. Although this animal was smaller than the others the ova were nearly mature. Adult female *Necturi*, 32.5 cm. in length, were examined before ovulation. In them cilia occurred in great abundance upon certain parts of the peritoneum. Cilia were constant in all the adult female *Necturi* examined from January to April on the following parts, viz., the hepatic ligament (Fig. 9), the ventral wall of the body cavity (Fig. 10), the membrane near the mouth of the oviduct (Fig. 11) and the serosa of the liver (Fig. 12).

On these parts of the peritoneum cilia were very generally distributed. Examinations were made at intervals over the surface and ciliated cells were found on every piece examined. Not every cell was ciliated, however. On the ventral wall ciliated cells were distributed from the pelvis to the cephalic end of the abdominal cavity. About one-half the number of adult female *Necturi* showed ciliated cells on the dorsal wall of the body cavity also. Where they occurred, they were distributed on the cephalic third of the dorsal wall and fewer cells were ciliated. No cilia were found in *Necturus* upon the mesentery, serosa of the ovary nor mesogastrium, although various writers have described cilia in these parts in the frog. Waldeyer ('70) spoke of cilia being present upon the mesentery of the frog, presumably in the female, as he mentioned cilia also upon the mesoarium. Thiry

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('66) found cilia upon the serosa of the frog's ovary, and Klein ('73) upon the mesogastrium, and Leydig upon the mesoarium. Ecker ('89) found cilia near the openings of the oviducts, and Grunau ('75) upon the serosa of the liver of the female frog and also near the oviducts, and Neumann ('75 upon the serosa of the liver of the female frog and in *Triton cristatus*.

In specimens of adult *Amblystoma punctatum*, taken just before ovulation, cilia were found upon the hepatic ligament (Fig. 13), the ventral wall of the body cavity, the membrane near the mouth of the oviduct, the serosa of the liver, the mesoarium and the fold of peritoneum supporting the oviduct, but none upon the dorsal wall of the body cavity. Adult female *Amblystomas*, taken soon after ovulation, as well as those taken in August, showed cilia in the same places as those taken before ovulation. Cilia were present in *Amblystoma punctatum* on the hepatic ligament and the serosa of the liver (Fig. 14) during the fall. This was determined by sections cut of a preserved specimen taken in October and killed in December. Very probably cilia occurred in other places, but it was impossible to see them in the sections. In male *Amblystoma* no cilia were found. No immature females were examined. From this it is evident that in *Amblystoma punctatum* cilia persist throughout the year.

In adult female *Diemyctylus viridescens* before ovulation cilia occur upon the hepatic ligament (Fig. 15), the ventral wall of the body cavity, the membranes near the mouth of the oviduct and the serosa of the liver. No cilia were noticed upon the dorsal wall. Neither immature females nor any females after ovulation were examined. Male *Diemyctylus* showed no cilia upon the peritoneum.

In adult female *Desmognathus* cilia were present before ovulation upon the hepatic ligament, the ventral wall of the body cavity and the membranes near the mouth of the oviduct, but not upon the dorsal wall of the body cavity. A young female with immature ova possessed no cilia upon the

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peritoneum. Two larval *Desmognathi* showed no trace of cilia, although serial sections were made of the whole body. The male *Desmognathus* had no cilia upon the peritoneum.

From the foregoing observations we see that, in all four species examined, cilia were always present in the adult female and that no cilia were found in the male or immature female, so far as examined.

Arrangement of Ciliated Cells.—Ciliated cells occur either singly or in groups between the non-ciliated cells. This was well shown in the fresh preparation. (Figs. 11 and 16.) The condition mentioned by Grunau of the ciliated cells being arranged in stripes radiating from the mouth of the oviduct, has not been noticed, although I found that the ciliated cells were most numerous near the mouth of the oviduct. (Fig. 11.)

Direction of Current.—To determine the direction of the current produced by the cilia, powdered carmine was placed upon the peritoneum immediately after the death of the animal. By the aid of a simple microscope it was seen to move towards the mouth of the oviduct and was finally drawn into the opening. Mrs. Gage's method of using minute blood clots instead of carmine was tried, and proved more successful than the carmine. Immediately after death a small blood clot was placed upon the peritoneum covering the ventral wall of the body cavity of an adult female *Necturus*. It traveled 10.5 mm. in ten minutes in the direction of the opening of the oviduct. Three hours after death a clot traveled only 6 mm. in ten minutes. In an adult female *Necturus*, where cilia were present on the dorsal wall, the direction of the current of the cilia on the dorsal wall also was in the direction of the mouth of the oviduct. Thiry demonstrated the direction of the current in the frog as being towards the mouth of the oviduct by using black pigment.

As the ciliary currents in *Necturus* are present only in the adult female and the direction of the current is toward the mouth of the oviduct, it seems to strengthen the theory that the cilia are to aid the ova in reaching the oviduct.

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The questions now arise when do the cilia make their appearance, are they persistent throughout the year and how are they developed? At present these are matters of speculation. To find out just when the cilia appear and how long they remain it would be necessary to examine a great number of specimens of all ages. As to their development there are various theories. Kolossow offers it as a suggestion that the irritation of the peritoneum, through the increase of the ovary in size about spawning time, may cause the appearance of cilia upon the peritoneum. Klein says that the "germinal cells in the female are ciliated," and Nicolsky calls the ciliated cells young epithelial cells. Schweigger-Seidel and Dogiel consider them as arising from the ciliated cells of the oviduct, while Grunau is not sure whether this is the correct view or whether the cilia are developed from the common flat epithelium of the peritoneum. Neumann holds this latter opinion, and Kolossow speaks of certain cells which are thicker than the others and covered with minute points which finally develop into cilia. This question I have not been able to decide. The ciliated cells are usually smaller but thicker than the others, but I could find no appearance of cilia just beginning to develop. There is little difference in the length of the cilia upon various parts of the peritoneum, although near the mouth of the oviduct they are generally longer.

STOMATES.

The subject of stomates has been much discussed ever since v. Recklinghausen first described them in 1862. Since that time many investigators have noticed them, both in mammals and the lower vertebrates. According to the best authorities stomates are small openings leading from the peritoneal or pleural cavities into the lymphatic vessels. They are described by v. Recklinghausen as being in the rabbit twice the size of a red blood corpuscle. Others merely say that they never exceed the size of an epithelial cell. They are usually surrounded by a radiating form of the

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epithelial cells. There may occur around the stomate a number of small granular cells, which Klein ('73) called "germinating cells." In the female frog he found that these cells possessed cilia. By Schweigger-Seidel and Dogiel ('66) the stomates are described as funnel-shaped, the smaller end being on the lymphatic side of the membrane. The supposed function of the stomates is to furnish a passage for the lymph from the peritoneal and pleural cavities to the lymphatic vessels.

A great diversity of opinion exists regarding the stomates. Many writers do not believe in their existence, but the prevailing opinion seems to be in favor of them. V. Recklinghausen ('63) and Dybkowsky ('66) demonstrated by experiments the presence of stomates in mammals, and Schweigger-Seidel and Dogiel ('66) in the frog. Ordmansson ('63) figures large and small stomates. Klein ('73) describes and figures stomates in the *septum cisternæ lymphaticæ magnæ*.

No doubt there is great danger of mistaking openings or precipitates for true stomates. Piersol said that sometimes the end of one of the processes of a connective tissue cell may protrude between the epithelial cells and, taking a brown stain with silver nitrate, be easily mistaken for a stomate. Such appearances he, with other writers, calls pseudo-stomates or stigmata. Toureux and Hermann ('76) said the so-called stomates are merely depressions in the membrane, which become filled with an albuminous liquid and so form a precipitate with silver nitrate. If such is the case, by thoroughly rinsing with distilled water these appearances may be avoided.

Stomates are especially abundant in the *septum cisternæ lymphaticæ magnæ* of the frog. This I have found to be the case in the female of *Rana Virescens* (Figs. 17-18). The cells are arranged in a radiating manner about the opening, the nucleus in nearly every case being situated in the end of the cell bordering on the stomate. No small cells are present around the opening. In *Necturus maculatus* there is

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no *cisterna lymphatica magna* and I could find no stomates anywhere in the peritoneum, although various methods of injection were tried and microscopical examinations of both the fresh and hardened material were made. Certain appearances (Fig. 19) I could not account for, but they are probably artificial.

FORM AND SIZE OF CELLS.

To bring out the boundaries of the cells v. Recklinghausen's silver method was adopted. Perfectly fresh material was used, as it was found that tissue which had been used for the examination in normal salt solution did not silver well, the salt and blood serum forming a precipitate with the silver nitrate, even though care was taken to rinse it well with distilled water before silvering. In membranes which had silvered in bright sunlight the lines of cell cement were sharp and dark, but when the process went on slowly in a dim light the effect was not so good as a brown precipitate was formed over much of the surface of the cells.

Ciliated cells stain more deeply than the non-ciliated (Fig. 8). Whether this is due to the presence of cilia or to albumen being retained between the cilia is at present unknown.

The cells of the epithelium vary greatly in form from the almost regular hexagonal shape of the cells of the serosa of the liver (Fig. 20) and testis (Fig. 21) of *Necturus* to the very serpentine form of the mesentery of *Diemyctylus* (Fig. 22). The number of sides varies from four to seven, the most common number being five or six. The difference in form is in a great measure due to the stretching or non-stretching of the tissue.

Schwartz, in an article in the *Anatomischer Anzeiger* for 1893, proved that this was so in the case of the peritoneal epithelium of the cat and the rabbit. He showed that when the organs were distended the epithelial cells of the peritoneum surrounding them were larger and of more regular outline than when the organs were empty. This was tried

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on the mesentery of *Necturus* and *Diemyctylus* by stretching and hardening the membrane and then comparing the form of the cells with those from part of the same membrane which had not been stretched. In the first case (Fig. 23) the cells were larger and quite regular in outline, while in the mesentery, which had not been stretched (Fig. 24), the cells were very sinuous in form and so contracted that they appeared smaller than the others.

There was no appreciable difference in the form or size of the epithelial cells between the male and female. In every case noticed in *Necturus* the cells on the dorsal wall of the body cavity (Fig. 25) were larger than those on the ventral wall (Fig. 10). Ciliated cells are usually smaller than the non-ciliated cells, but thicker, so that they project more than the others (Figs. 9 and 10). In *Necturus* the cells of the serosa of the liver and other solid organs are more regularly hexagonal than on any other part. Probably this is due to the fact that these organs are more uniform in shape and size than the others. The shape of the cells of the hepatic ligament, serosa of the liver, mesopneumon and membrane between the spleen and lungs and between the liver and lungs are shown in Figs. 26, 27, 28, 29 and 30.

Measurements.—In length the cells averaged 57.3 μ., in breadth, 39.8 μ., while the average thickness was 12.31 μ. The average size of the nuclei near the mouth of the oviduct in *Necturus* was, length, 38 7 μ., width, 22.5 μ., and thickness, 8.75 μ. The average length of the cilia upon the hepatic ligament in the female *Necturus* was 3 12 μ. and of those upon the membranes near the mouth of the oviduct was 3.75 μ.

NUCLEI.

The nuclei are usually large, oval or kidney-shaped. They are nearly always placed eccentrically and generally the nuclei of two adjacent cells are in juxtaposition. The cells project opposite the nuclei. In the epithelium of the peri-

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toneum, covering the contracted stomach and intestine of *Necturus*, a peculiar condition was noticed. The rows of cells seemed to be arranged in pairs. In each pair the nuclei were juxtaposited so that there was the appearance of the nuclei being situated in double rows (Fig. 31). But when the organ was distended this appearance was lost (Fig. 32). Occasionally a cell with two nuclei was noticed (Fig. 33).

SUMMARY.

The result of this investigation may be summed up as follows:

1. The peritoneum is made up of two parts, viz., the connective tissue stroma and the epithelium.

2. The connective tissue stroma is composed of white connective tissue, elastic fibers and bundles of smooth muscle cells.

3. The peritoneal epithelium was ciliated only in the adult females studied. It was impossible to demonstrate cilia in the males or young females, although their presence upon the peritoneum of some *Amphibia* has been described by a few writers.

4. All the specimens of *Necturus*, *Desmognathus* and *Diemyctylus* were taken from January to April and none were examined after spawning. Specimens of *Amblystoma* were examined both before and shortly after spawning and in August, and one specimen which was taken in October and prepared in December.

5. In the adult females of all the species named taken in the spring and in August, cilia were constant upon the following parts: Hepatic ligament, the ventral wall of the body cavity, the membranes near the mouth of the oviduct and the serosa of the liver.

6. In *Necturus* some of the adult females showed cilia also upon the dorsal wall of the body cavity.

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7. Specimens of adult female *Amblystomas* taken in the spring and in August differ from the other species in having cilia upon the mesoarium and the membrane supporting the oviduct.

8. In a single example of adult female *Amblystoma* taken in October and prepared in December, cilia were present upon the hepatic ligament and the serosa of the liver.

9. The direction of the current produced by the cilia is toward the mouth of the oviduct.

10. As indicated by the current the function of the cilia is probably to carry the ova to the oviducts.

11. Blood clots were carried by the cilia on the ventral wall immediately after death at the rate of 10.5 mm. in ten minutes, and three hours after death at the rate of 6 mm. in ten minutes.

12. No stomates were found in *Necturus maculatus*.

13. The shape of the cells varies greatly, but most of this variability is due to the stretching or non-stretching of the tissue.

14. The size of the cells varies in different parts of the peritoneum and in different species.

15. The cells on the dorsal wall of the body cavity are usually larger than those on the ventral wall.

METHODS.

The specimens were killed in 1-6 chromic acid, ether being added to the proportion of 5 c.c. to the liter. The abdomen was then opened and the various parts of the peritoneum were examined for the presence of cilia. The tissues were kept moist with blood serum or normal salt solution.

Bits of clotted blood or finely powdered carmine were used to determine the direction of the ciliary currents.

Other specimens which had not been utilised in the above

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manner were treated with silver nitrate. After the abdomen was opened and the specimen thoroughly washed in distilled water to remove the blood and lymph, a 1-5 per cent. solution of silver nitrate was poured over it and the viscera carefully moved so as to allow the silver to reach every part of the peritoneum. This was permitted to remain two or three minutes, or until the peritoneum had a silvery cast. Then after several washings with distilled water the specimen was placed in a porcelain dish with plenty of distilled water in the direct sunlight or the strongest daylight until the peritoneum was of a light brown color. In the bright sunlight only a few minutes were required.

For a nuclear stain Gage's hematoxylin (Gage, 1892,) was the most successful. In preparations showing the face view of the epithelium no stain beside hematoxylin was used. In the sections eosin was used as a counter stain. For the study of the connective tissue, the membrane was gently penciled to remove the epithelium. A 2 per cent. acetic acid caused the white connective tissue to swell up and become transparent, so the elastic fibers which were unaffected by the acid stood out plainly. Van Gieson's picric acid fuchsin (Freeborn, 1893,) was used to stain the white connective tissue.

The tissue was hardened simply in alcohol, 50, 67 and 82 per cent. being used, the time for the first two being one to three days and the tissues were left in 82 per cent. until needed. Picric alcohol 1 to 3 days and then 67 and 82 per cent. alcohol was also used, but the picric acid tended to obscure the cilia.

Hardened tissues were mounted in balsam and glycerine jelly was used for the fresh.

Both the paraffin and collodion methods were used in imbedding and sectioning.

The larval forms were hardened, some in Fish's picro-aceto-sublimate, and others in formalin and imbedded whole in collodion and sectioned.

For the dissociation of the epithelial cells various reagents

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were tried, but equal parts of Gage's picric alcohol and water, 2 to 4 hours, proved the most successful.

To determine whether the change in the form of the cells was due to the stretching of the membrane the following method was employed. Pieces of the fresh mesentery and hepatic ligament of *Necturus* were stretched over Hoggan's histological rings, treated with silver in the usual way and hardened. The mesentery of *Diemyctylus* being too small to cover a ring, the necks of small homeopathic vials were used for this instead of rings. The small intestine of *Necturus* was stretched by distending it and then hardening.

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PLATE I.

Fig. 1. Transection through the cephalic part of the abdominal cavity of *Necturus maculatus*, showing the peritoneum and its relation to the body wall, liver, stomach, lungs and oviduct.

a. — white connective tissue fibers.
b. — elastic fibers.
c. — pigment cells.
o. — oviduct.
s. — spleen.
i. — small intestine.
e. — lung.
ov. — ovary.
p. — peritoneum.
st. — stomach.
v. — postcava.
x. — dorsal aorta.
y. — portal vein.
l. — liver.

Fig. 2. Transection through the body cavity of *Necturus* farther cephalad than Fig. 1.

Fig. 3. Transection through the body cavity of *Necturus* at middle of small intestine.

Fig. 4. Fresh mesentery of small intestine of *Necturus maculatus*. Epithelium removed by penciling.

Fig. 5. Bundle of smooth muscle cells from mesentery of small intestine of *Necturus maculatus*.

Fig. 6. Isolated smooth muscle cells from mesentery of small intestine of *Necturus maculatus*.

Fig. 7. Peritoneum from dorsal wall of body cavity of *Necturus maculatus*. Fresh, epithelium removed by penciling.

a. — ciliated cell.
b. — non-ciliated cell.
c. — cell folded over, showing cilia in profile.

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PLATE I.

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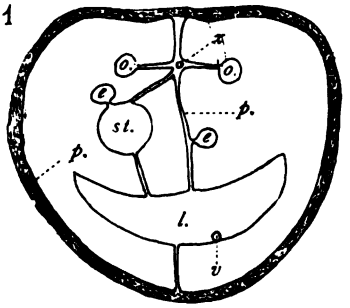

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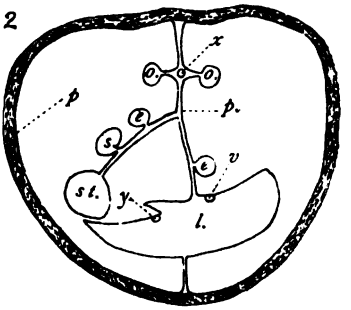

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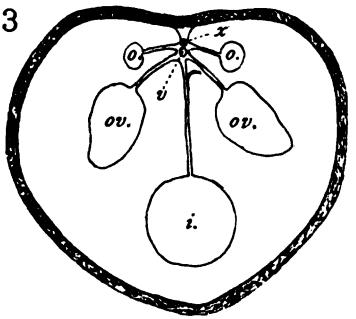

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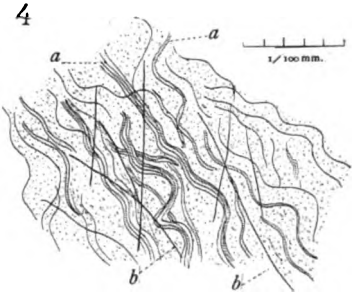

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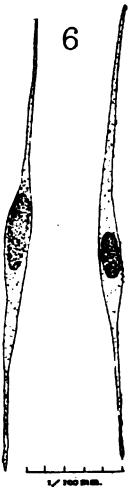

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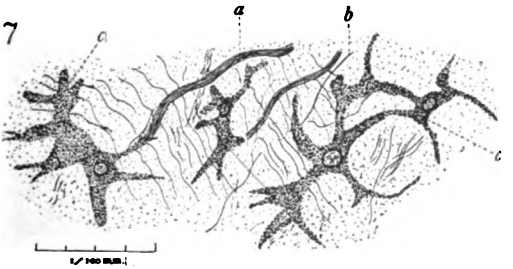

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PLATE II.

Fig. 8. Cross section of the serosa of the liver of male *Necturus*, showing absence of cilia.

Fig. 9. Cross-section of the epithelium from the hepatic ligament of adult female *Necturus*, showing cilia.

Fig. 10. Surface view of peritoneal epithelium from the ventral wall of the body cavity of adult female *Necturus*.

Fig. 11. Surface view of fresh peritoneal epithelium near mouth of oviduct of female *Necturus*.

Fig. 12. Cross-section of epithelium from liver of female *Necturus*

Fig. 13. Cross-section of hepatic ligament of adult female *Amblystoma*, taken just before spawning.

Fig. 14. Cross-section of serosa of liver of adult female *Amblystoma*, taken in October.

Fig. 15. Surface view of epithelium from hepatic ligament of female *Diemyctylus*.

Fig. 16. Surface view of fresh peritoneal epithelium from hepatic ligament of female *Necturus*.

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PLATE II.

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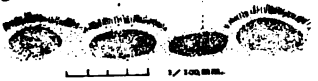

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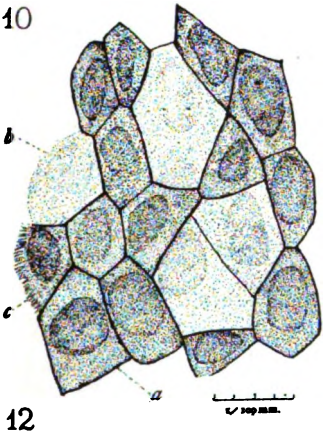

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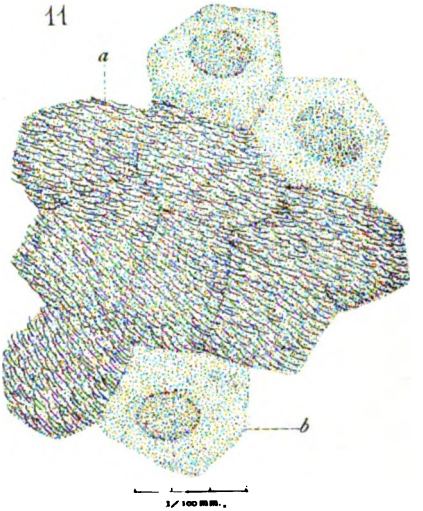

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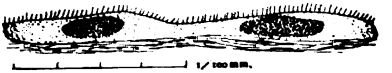

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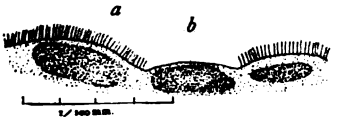

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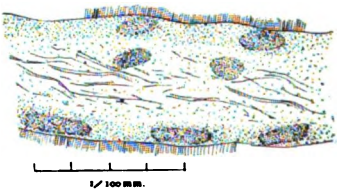

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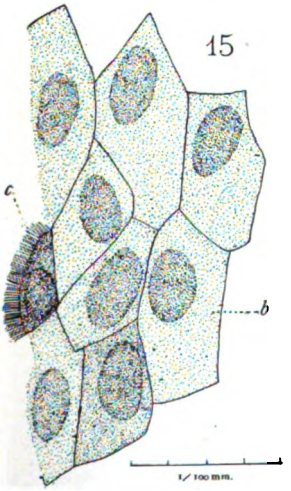

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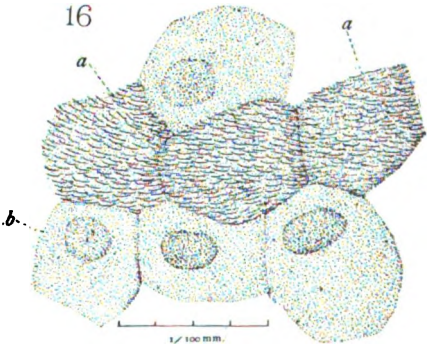

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PLATE III.

Fig. 17. Peritoneal side of *septum cisternæ lymphaticæ magnæ* of *Rana virescens*.

a. — stomate.

Fig. 18. Lymphatic side of *septum cisternæ lymphaticæ magnæ* of *Rana virescens*.

a. — lymphatic opening of stomate, shown in Fig. 17.

Fig. 19. Peritoneum from dorsal wall of body cavity of male *Necturus*.

a. — appearance not accounted for.

b. — opening between epithelial cells, probably artificial.

Fig. 20. Face view of epithelium from ventral surface of liver of male *Necturus*.

Fig. 21. Face view of epithelium from testis of male *Necturus*.

Fig. 22. Face view of epithelium from mesentery of small intestine of female *Diemyctylus*.

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PLATE III.

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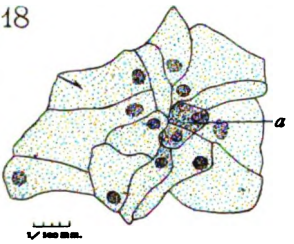

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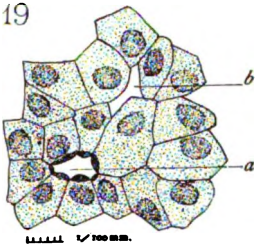

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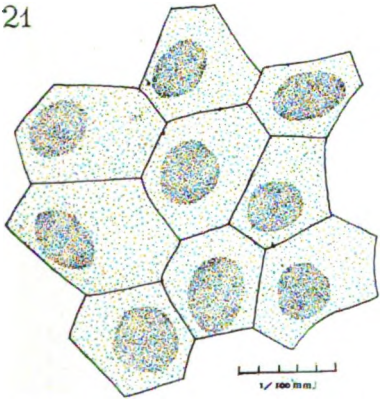

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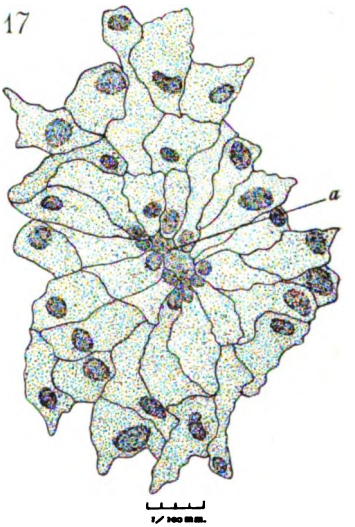

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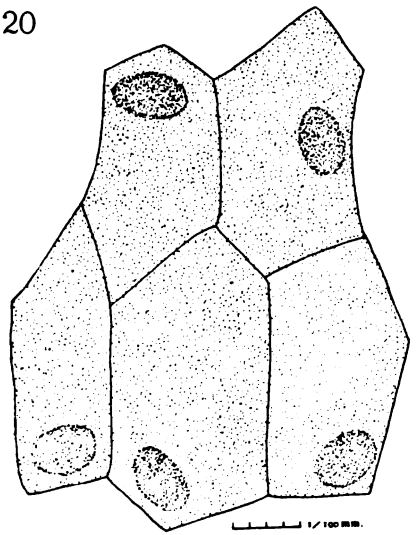

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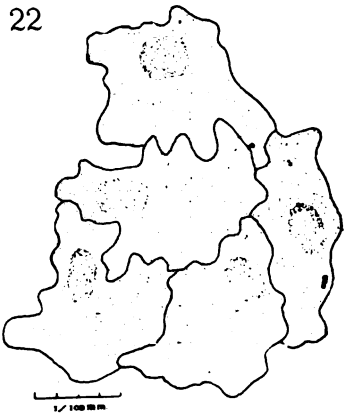

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PLATE IV.

Fig. 23. Face view of epithelium from the stretched mesentery of small intestine of male *Diemyctylus*.

Fig. 24. Face view of epithelium from the unstretched mesentery of small intestine of male *Diemyctylus*.

Fig. 25. Face view of epithelium from the dorsal wall of the body cavity of male *Necturus*.

Fig. 26. Face view of epithelium from the hepatic ligament of female *Necturus*.

Fig. 27. Face view of the epithelium from the mesopneumon of male *Necturus*.

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PLATE IV.

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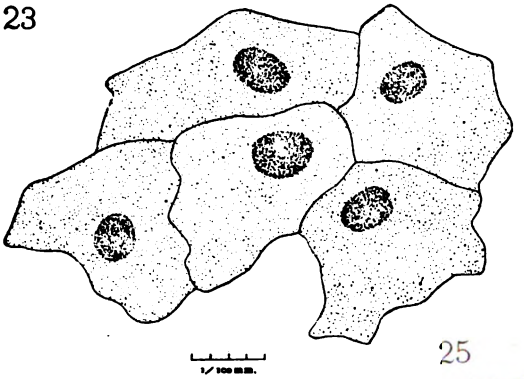

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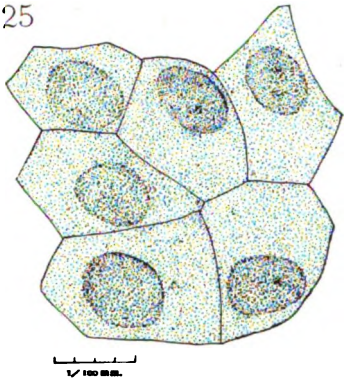

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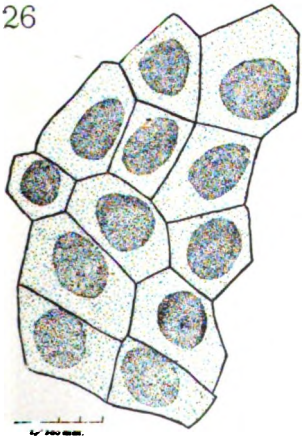

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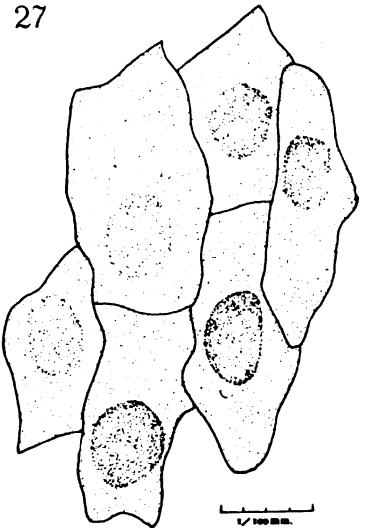

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PLATE V.

Fig. 28. Face view of epithelium from the membrane between spleen and lungs of male *Necturus*.

Fig. 29. Face view of epithelium from the liver of male *Desmognathus*.

Fig. 30. Face view of epithelium from the mesopneumon of female *Diemyctylus*.

Fig. 31. Face view of peritoneal epithelium from the contracted small intestine of male *Necturus*.

a. — peculiar arrangement of the nuclei.

Fig. 32. Face view of the peritoneal epithelium from the distended small intestine of the male *Necturus*.

Fig. 33. Epithelial cell from the hepatic ligament of the female *Necturus* with two nuclei.

Fig. 34. Isolated epithelial cells from the hepatic ligament of male *Necturus*.

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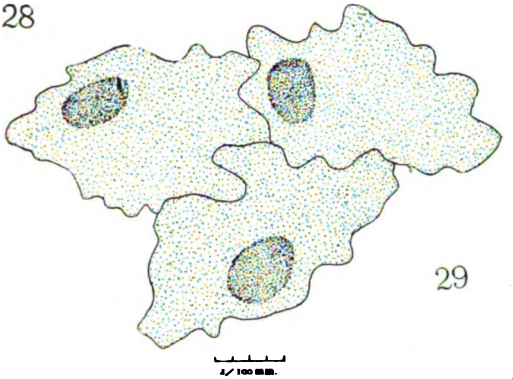
PLATE V.

28



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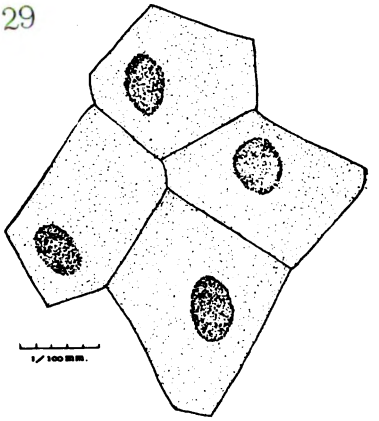

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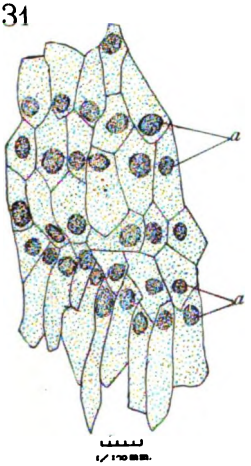

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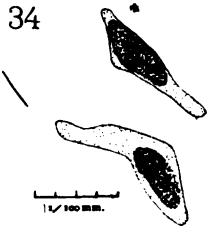

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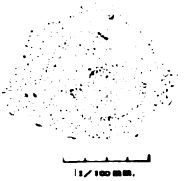

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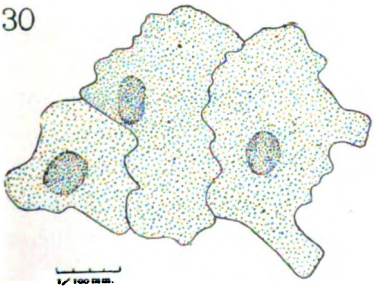

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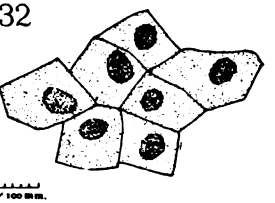

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PHOTOMICROGRAPHY.

THOMAS J. BRAY, WARREN, O.

The subject of the paper assigned me, as you will see by the program, is Photomicrography by the Use of Ordinary Objectives, Practically Considered. The primary object of the paper is to show what can be done with such objectives as are to be found in the outfit of a physician or amateur, and to aid, as far as possible to simplify and popularise this beautiful process. The theme being a practical consideration of the art, it precludes the idea of a scientific discussion of the subject, hence I shall, in very plain words, give you a brief description of the methods which I have found best suited for this work. I will also narrate some of my failures, trusting they will act as a warning to those who may be working along this line, in order that they may avoid similar errors. With these introductory remarks, I ask your kind attention to the subject matter of the paper.

A photomicrograph has been defined briefly and properly as a "Macroscopic photograph of a microscopic object," in contradistinction to a microphotograph, which means "a microscopic photograph of a macroscopic object." I will make these terms a little plainer by saying that a photomicrograph is a photographic enlargement of a minute object, and a microphotograph is a photographic reduction of a large object. By impressing upon your minds the fact that a photomicrograph is an enlargement and a microphotograph a reduction of the objects by photography, then these terms will not be as liable to be misused as they sometimes are. For many years I labored under the impression that in order to be successful in the production of photomicrographs, I

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would have to use objectives that were constructed for that special purpose, that the chemical, optical and visual foci should be coincident. I found, in reading up on this subject, that most writers so express themselves; some of them go so far as to give directions how to get a general average of these foci. They advise to work by trial and error, thus: to expose a plate slightly nearer the object than the visual focus, then another slightly farther away, and in this way find the proper location for the sensitive plate.

Recent works on photomicrography, notably Professor Gage's book on Microscopic Methods, states that good photographs can be made by the use of the ordinary objectives. He gives some very practical advice on this subject. I made many trials as per instructions given in the text-books, but the results were a hazy, out of focus, unsatisfactory negative. I sought the advice of some of my friends whom I supposed to be masters of the art, but I got the invariable answer, "that to be successful I must use objectives specially corrected for that purpose." I failed to see *why* I should not be able to get just as good an image on the plate as I could get on the focusing screen, provided the plate and screen were exactly in the same plane. I so expressed myself and was as often told, "that when I knew more about the subject, I would understand the reason why corrected objectives were a necessity." These expressions from experts only made me more eager to succeed according to my views and judgment as an amateur photographer of many years practice. I persevered, however, making many scores of failures, when at last I made this discovery, that I was beginning at the wrong end—using high power objectives before I had learned to work with the lower powers. I reasoned thus: that high power objectives, such as 1-8, 1-10 or 1-15, gave a small field and poor light, and that they required the nicest and finest adjustments, the greatest skill, the very best optical appliances and apparatus to operate them, and the most effective light for their illumination that could be obtained. The low powers, on the other hand, gave a larger field, the result

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being a small picture. It then dawned upon me that what was required for successful work was to enlarge these small pictures by the use of a long camera, that is, to work upon the principle of the photographer's enlarging process rather than by the use of high power objectives. These views I have proved by many practical demonstrations, to my own satisfaction at least, to be correct.

I shall now take up the subject of apparatus, lighting, focusing and finishing of the negative, giving the details of the work as I practice them. The apparatus consists of a long bellows camera. This may be made of several sections of light-tight pine boxes, blackened inside to eliminate all reflections from the sides, and made to slide in each other, having a focusing screen and a plate holder attachment on the end of the rear section, and an arrangement at the front end section for securing the tube of the microscope to it. This may be a slip tube, or a black bag, or any simple connection that is perfectly light-tight and capable of contraction and expansion to suit the movement of the microscope tube in focusing the object, or preferably a camera made specially for this work may be used. If large negatives are to be made, I would strongly advise doing away with the camera altogether and use a dark room to serve the purposes of the camera, the microscope and light being on the outside and the tube of the microscope fitted to a door or shutter, thus shutting out all light from entering the room save that which comes through the objective. On the inside of the dark room construct a rack for carrying a movable piece suitable for holding the focusing screen and plate holder. This would be a very cheap and complete arrangement. The operator would, of course, stand inside the camera or dark room. A microscope is not a necessity ; any arrangement that will hold the slide centrally and permit of the light passing through the object will answer the purpose. I use a microscope simply because I have one and on account of its convenience in making necessary adjustments and focusing.

The operation of picture-making is as follows : The

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tube of the microscope is turned down so that it lies in a horizontal position; it is then attached to a short bellows and it, in turn, to the front of the camera. The substage bar and mirror bar is swung around out of the way. A Welsbach incandescent gas burner is placed back of the stage about ten inches distant, with the bright white flame located centrally. About midway between the gas jet and the stage is placed a bull's-eye condenser with the flat side towards the object, and by placing a piece of white paper on the stage the light is centered upon it; that is, I focus the bull's-eye by moving it to or from the light so as to get the best even clear light possible. When this is done the paper is removed and the slide to be photographed is placed in its position on the stage. Some prefer a mechanical stage, but as they are expensive I could not afford this luxury, but have managed to get along without one by using Bausch & Lomb's glass stage; its movements are easy and after a little practice I find it answers all my purposes. The objective is now put in place and run down to about the working distance. The object can now be seen on some part of the ground glass screen, perhaps indistinctly; when observed it should be focused by the unaided vision and the object centered carefully on the ground glass. If the circle of light is out of the center, then the camera and the microscope are not in line. Adjust them by moving the tube end of the microscope to the right or left, up or down, as required and shown by the circle of light on the ground glass. If the circle of light is not as large as the object, then an objective of lower power must be used. With a six-inch to three-inch variable I have a considerable range of field and amplification, and with a six-foot bellows extension to my camera, I have under my control a great variation in amplification, besides being able to photograph many large objects up to five-eighths of an inch in diameter. Objects that are larger than this, I take with an ordinary photographic camera. The focusing arrangement used is an extension to the pinion shaft; it is attached to the milled head and extends beyond the camera a couple of

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inches; on the end of this extension a loose lever, having a tightening screw in its hub, is placed, the lever extending down to within half an inch of the table. To the lower end of this lever a piece of stiff wire is fastened, the wire being as long as the camera bed, and is held in place by passing through eyes screwed into the bed-piece of the camera. This focusing device was invented by Dr. Mercer, and as I have not heard of his patenting this device, it can be used, presumably, by all who wish to do so. By the use of this attachment, the utmost nicety of adjustment can be made. After focusing carefully by the unaided vision, the thumb screw is tightened in the hub of the lever, then, by moving the wire rod to or from the object, a very fine focus can be obtained. With this arrangement I can focus a one-eighth water immersion objective by exercising a little care, as the slightest movement of the wire reduces the movement of the tube about one-twentieth of the motion given to the rod. Consequently there is no occasion to use a fine adjustment at all. The focusing screen is the ordinary ground glass screen, with Mr. Walmsley's improvement thereon. This consists of cementing with balsam, cover glasses, at several points around the limits of the picture and one in the center, all on the ground side of the screen, thus showing clear glass at these points. This is a very good focusing device, in fact, the best I have tried.

It is used thus: First focus by the eye, as nearly correct as possible, on the ground glass part, then with a two-inch eye-piece, used as a focusing glass, focus sharply on the cover glasses. If an object is of uneven surface, then focus for the part that is required to be shown clearly, or take a general average of the whole object. Take, for example, the fly's tongue. This object has an uneven surface; hence, if the focusing is done upon the hairs on the false trachea, then the spirals will not be so sharp; if we focus on the spirals, then the hairs will be slightly out of focus; therefore, in such cases, a general average focus must be taken to show the object as a whole. The objectives I

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use for photomicrography are Bausch & Lomb's student series, from one-eighth down to six inches. These will answer every purpose of the non-professional or amateur. One of the most useful objectives that I know of is one made by them. It is a six-inch to three-inch variable; it has a very good definition, and, by turning a milled collar, any range of amplification can be obtained within range of the objective, and with this lens and a long camera a great variety of work can be done. It is particularly useful for histological specimens, as it will light the whole of an object, provided it is not over five-eighths of an inch in diameter. For subjects larger than this, I resort to the ordinary photographic method of copying and enlarging, as before stated. For example of that kind of work, I point you to the photograph of the firefly, which is exhibited for that purpose. To summarise, first adjust the light, then put on the lowest power objective that will give the amplification desired, and that will also give a circle of light extending beyond the outer edges of the object. Extend the camera until the picture is of the size wanted, focus again, then attach and use the lever focusing-device before mentioned. Focus sharp on the cover glass spots, using a two-inch eye-piece, or a focusing glass for this purpose. When this is done, put in the color screen between the bull's-eye condenser and the stage of the microscope and expose about three or four times as long as you would with the same light and conditions without the screen. The screen used is one made by Bausch & Lomb for cloud photography, and it belongs to my 8 x 10 view outfit. I have it rigged up on a stand for the purpose herein named. It is a most useful affair for all objects that have a yellow tinge in the balsam or the object. It is indispensable with me for many objects; for example, the photographs of arranged diatoms on some of the souvenir cards, also on the large photographs in the frame, where some are very transparent, others not so transparent, and some are quite dense. The use of the screen seems to have the same effect on such objects as it has upon the clouds in a

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landscape view. It in a great measure evens up, as it were, the different lights in the object, that is to say, it retards the high lights, which are apt to be over-exposed, to a greater degree than it does the middle lights and the shadows. Many of the diatoms in the picture shown would not be seen at all, were it not for the effect of this bi-chromate cell. I will here relate an experience I had previous to using this screen: My friend and co-worker, Mr. Harry G. Carter, of Minneapolis, sent me a slide of the proboscis of a large blue-fly, the one pictured on some of the souvenir cards, and the one shown in the large picture exhibited. I tried to photograph this slide and made over a dozen failures. I wrote to Mr. Carter that I could photograph a slide that I owned and make a success every time, but that I utterly failed to make a single picture from his slide; that the best results I could obtain was an "out of focus, hazy" kind of a negative. He replied that my lenses were not suited for the work, and that I could not expect to succeed with the objectives I was using. I answered him that with another slide I had, of the same object, I could get a good picture every time I made an exposure, hence I could not agree with him. Soon after this I tried the color screen and made a success of it at the first trial. This convinced me I was right, that the fault was outside of the objective. To still further test this matter, I bought a one-inch "no name" objective in a "spectacle shop" in Cleveland. It cost me $4.00 with a brass box. I bought it because it was a cheap affair and certainly could not have been specially corrected for photography. I tried this objective and made some splendid good sharp pictures with it. I have it with me, also a specimen of the work it has done. Should anyone present be curious to examine them, they can do so at the close of this session.

The sensitive plate will now be considered briefly. I find in my practice that almost any of the standard make of plates will give good results. I have used Seed's, Cramer's, Eastman's, Stanley's, Carbutt's, Hammer's and many others, and

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got along very nicely with all of them; yet I have a decided preference for Cramer's slow isochromatic plates. They are a little slow, but they are much easier to work with than the more rapid ones. With the Welsbach incandescent light and a low power objective, using Cramer's crown plates without a color screen, the exposure must be made in about a half second or the plate will be spoiled from over-exposure. Of course, much depends on the character of the object. I would strongly advise slow plates, especially for those not well up in the operations of photography.

After the exposure comes the developing. This is done in the same way, and by the same reducing agents, as is practised by the ordinary photographer. There are a number of good mixed developers made, and in the photographic journals may be found the formula for compounding them. They all have merit. I can specially recommend Mr. Walmsley's "Universal Developer" as being very suitable for this purpose. I use a compound known as "Monarch Developer;" it is prepared and sold by W. S. Bell & Co., of 435 Wood street, Pittsburg. It is a universal developer, suitable, when diluted, for negatives, transparencies, lantern slides, bromide paper, and the like. It is a very clean, clear compound, and can be used over again repeatedly. It is composed of two reducing agents—namely, hydrochinon and para-amido-phenol. I have used it for several years and can fully recommend its use as a developer. The fixing bath that I consider best is an acid bath, with chrome alum as a hardener. This I make according to Cramer's or Carbutt's formula. It clears, fixes and hardens, and is used over again until too weak to be effective; it is then thrown away and new bath made. After the plate has been fixed and cleared it is thoroughly washed, preferably in running water, for one hour to eliminate the last trace of the hypo left in the film by the fixing process.

The negative is now to be carefully examined. If it is found to be dense enough to print from, it is then laid on a rack to dry. If it appears thin, then intensifying must be

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resorted to to bring up the density or opacity of the high lights, after which it is washed well and laid aside to dry. When dry and hard, wipe the film side with a rag dipped in alcohol; this hardens the film and cleans it off smooth and clear. Some think that a negative when dry and hard is ready to print from, but this is a mistake. There is a great deal of "doctoring" that can be done to a photomicrograph negative that will improve the appearance of the resultant positive picture. Very often we have a negative that has very little difference in the printing qualities between the object and the balsam in which it is mounted. In such cases the removal of the background is the operation to be performed for its improvement. This is done by scraping away all the film up to and close around the object, as shown by the black background in the picture of "fetal hand" exhibited. If a clear white back is desired, then the film is painted over with "Gihon's opaque," a pigment made for the purpose. This effect is also shown in several of the pictures exhibited and in some of the souvenir cards. Sometimes pin holes are found in the film or a scratch from careless handling; these should be painted out with either Prussian blue water color or India ink, and these spots or scratches should be made of the same opacity as the surroundings. Very often the circle of light does not extend far enough to allow the print to be trimmed as it should. In that case a mat is used. This is a piece of opaque paper with the center cut out in a circle, ellipse or any other shape to suit the outline of the object. This feature can also be noticed on some of the souvenir cards. I like to use a mat because I can make a record on the margin of it, giving the exposure, objective and plate used, with date and the like. This mat I stick on to the glass side of the negative with mucilage and it always remains there.

We now come to the printing of the positive, and after trying all kinds of papers to be procured here and some from other countries, I prefer the "Platinotype" to any, for the reasons which follow: It is the busy man's paper, because it

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is easily and quickly manipulated; it is not sticky or soft; it does not require the use of ice and a thermometer in the bath to prevent an attack of "green sickness or yellow fever;" also because I think it more artistic for photomicrographs than shiny, glossy paper; and lastly, every piece will make a picture sure and without the risk of losing any. This paper is expensive, but the working of it is so easy, simple and satisfactory, that if once tried it will surely meet with favor.

The operations are as follows: Print until the picture is bronzed in the shadows. When ready to develop put it in a tray or on a pane of glass and brush the developer over it; the picture comes out instantly; in a few minutes it is ready to be rinsed in two or three successive baths of water acidulated with muriatic acid, then washed for twenty minutes in clear water, after which they are hung up to dry, and the job is done and you have for your trouble a thing of beauty and a joy forever. They can then be mounted in an album or on cards, as may be desired.

In conclusion I will say that I advise persons having no knowledge of photography to practise the same with a view camera before commencing to make microphotographs. The knowledge so obtained will be very helpful in the prosecution of photomicrography.

I shall be pleased to answer any questions that anyone may wish to ask me, or to describe more fully any point that I have not made clear. I will also explain by letter any point of difficulty that may be met with in practice, should such occur. Address me at Warren, O., and it will receive my best attention.

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SYSTEMATIC PHOTOMICROGRAPHY AND APPARATUS PERTAINING THERETO.

By JAMES B. SHEARER, Bay City, Mich.

Although much has been written upon this most interesting subject, and many papers appear each month in the scientific journals, it may seem presumptuous for me, an amateur, to say that not enough stress has been laid upon the necessity for systematic work in photomicrography, in order to obtain the best results.

Too often the worker depends upon guesswork in timing exposures; is careless in arranging the light so that the field is unequally illuminated; gives little thought as to the kind of photographic plate used; develops with any developer that is handy or cheap; in fact, pays little attention to the details that are necessary to produce fine negatives.

Having once made a correct exposure, one ought to be able at any time to duplicate it, or make an approximately correct one for any similar object, under the same or similar conditions and combination of lenses and light. It is for the purpose of teaching beginners, or any who are willing to be taught, how this may be accomplished that the writer has presented this short paper.

Experience, and many failures have fully demonstrated that much valuable time would have been saved, and often great annoyance avoided, if proper data of former exposures had been preserved and at hand in convenient form for ready reference. Suppose one wished to make a photomicrograph of a histological slide stained with borax carmine, using a one-fourth objective, two-inch eye-piece and a magnification of 300 diameters, and when all had been arranged to the

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satisfaction of the operator it was found that the photographic plate was thirty inches from the slide, the lamp fourteen inches, and the Abbe condenser, with pin-hole stop, one-eighth inch from slide; how simple it would be to look over previous data and find an exposure of another slide, stained red, and same combination of lenses and light used. We would then have a sure guide as to time of exposure, and even with different lenses one would have an approximate guide.

With this object in view, some years ago, the writer had a book of forms prepared to enter and preserve such data, which is as follows:

No.....Name.....magnified.....diameters with .....objective and.....Eye-piece.....and..... inch draw tube; using.....light at.....inches. Bull's eye condenser at.....inches and.....condenser with.....and.....stop at.....inches from slide. Exposed.....seconds, minutes on.....plates No.....at.....inches from object. Color of object ..... Date..... Remarks.....

The advantage to the worker in using such a book of data is very great, saves much time, not only in making exposures, but also in determining magnifications; for, once determined, they are always the same for the same combination of lenses at the same distance of the plate from the object.

Fairly good photomicrographs can be made with ordinary objectives, but the best work requires the finest lenses, correct manipulation of the light, proper kind of photographic plate, right time of exposure and care in the development.

Perhaps the writer can do no better than to describe in detail the method of making a photomicrograph with the apparatus figured in Plate I.

The object to be photographed having been placed upon the stage of the microscope, and the latter turned down

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horizontally to the position shown in Plate II., the operator seats himself and views the object through the microscope, centers it, arranges the light so that the field of view is equally well illuminated, focuses and, if necessary, adjusts the objective so as to obtain the best visual image possible, showing the detail he wishes to photograph.

Then, by means of the turn-table shown at L in Plate II., the microscope is swung toward and connected with the front board of the camera by pressing the front board back until the tube of the microscope is in position to pass through, when released, the opening prepared for it in the camera front.

The focusing string for fine adjustment is then passed over the milled head of the fine adjustment wheel of the microscope and placed in the groove prepared for it on the wheel, the camera back is extended until the desired amplification is obtained, and fastened by means of a set screw. Next the focus is corrected, for distance from object, by means of a focusing glass held against the screen of the camera and through which the operator looks at the image on the screen of the camera while he adjusts the focus, by means of the focusing rod, D, shown in Plate II., which extends the full length of the camera bed. The greatest care should be taken that the side of the screen on which the image is focused lies in exactly the same plane that the sensitive film of the photographic plate will occupy when placed in the plate-holder and the latter adjusted on the camera; the plane of the focusing screen should also be at an exactly right angle to a straight line drawn from its center, through the center of the microscope tube, to the center of the source of illumination.

When the operator is satisfied that all the arrangements are as perfect as he can make them, he notes in the book of forms the full data, and determines from previous data of exposure, that has proven to be correct, the time to expose the plate. The correct exposure depends upon many circumstances: kind of light, distance of light from object, dis-

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tance of photographic plate from object, sensitiveness of plate and many others.

The time of exposure having been determined, the light is shut off from the object by means of a screen placed on the sub-stage condenser-arm of the microscope, the plate-holder placed in position and slide withdrawn. The operator, with watch in hand, removes the screen and exposes the plate for the exact time, as predetermined from data-book of exposures.

With this system one can always make a second exposure, if found necessary, giving a little more or a little less time, as required, and, keeping in mind the law of light, "The intensity diminishes as the square of the distance," one can calculate just what exposure to give for different magnifications, with the same combination of lenses, provided one always works with the same light.

The next step is to develop the plate. It is very important that a good developer be used. One of the best, the writer has used, is that of Dr. William M. Gray, of the Army Medical Museum, Washington, and is made up as follows:

No. 1.—Hydrochinon ..... 96 grains.
Soda sulphite (crystal) .....480 "
Water .....8 oz.

No. 2.—Soda carbonate .....400 grains.
Water .....8 oz.

To develop take four parts of No. 1, two parts of No. 2 and sixteen parts of water. It is a slow developer, but gives great contrast and density. If developing is done in warm weather make up developer with ice-cold water and keep ice in the clearing bath. Carry the development far enough; most negatives are taken out of the developer too soon; the exact time must be learned from experience. After the plate is fully developed, wash and place it in Carbutt's acid, fixing and cleaning bath until all trace of the silver has disappeared, then in running water for one hour. For general work, the

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writer has had the best success with the Carbutt orthochromatic plate, No. 23; and, when used with gaslight, no change of focus is necessary on account of visual and actinic focus.

Many are deterred from working at photomicrography on account of the prevailing idea that the necessary apparatus is very expensive. Like all other branches of study and recreation, it may be made very expensive, but really good work may be accomplished with the cheaper apparatus, if properly manipulated.

The apparatus, as shown in Plate I., excepting the microscope, can be had for less than $60, so that the claim of expense ought not to prevent such an outfit being in every institution of learning in this country, where the use of the microscope is taught. A brief description of this apparatus will not, perhaps, be amiss:

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PLATE I.

Represents the entire apparatus in position for making an exposure.

a. —camera back, carrying plate holder and focusing screen.

a'.—camera front.

b. —camera front extension with circular opening for microscope tube.

c. —wood support into which curtain pulley is fastened, over which the upper loop of fine adjustment focusing string passes.

d. —focusing rod extended full length of camera bed.

e. —spool on end of focusing rod over which the lower loop of fine adjustment focusing string passes.

f. —bull's-eye condenser.

g. —Welsbach gas light.

h. —support for microscope stand, around which the lamp-carrier can be revolved so as to obtain suitable light for opaque objects.

i. —lamp-carrier with scale to show distance of light from object.

j. —stand supporting camera.

k. —sliding support for turn-table with scale to show the distance turn-table is from normal position.

l. —turn-table carrying support for microscope stand lamp and bull's-eye condenser.

m. —curtain pulleys over which focusing string from coarse adjustment wheel passes on its way to rear of camera stand.

mi.—microscope stand.

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PLATE I.

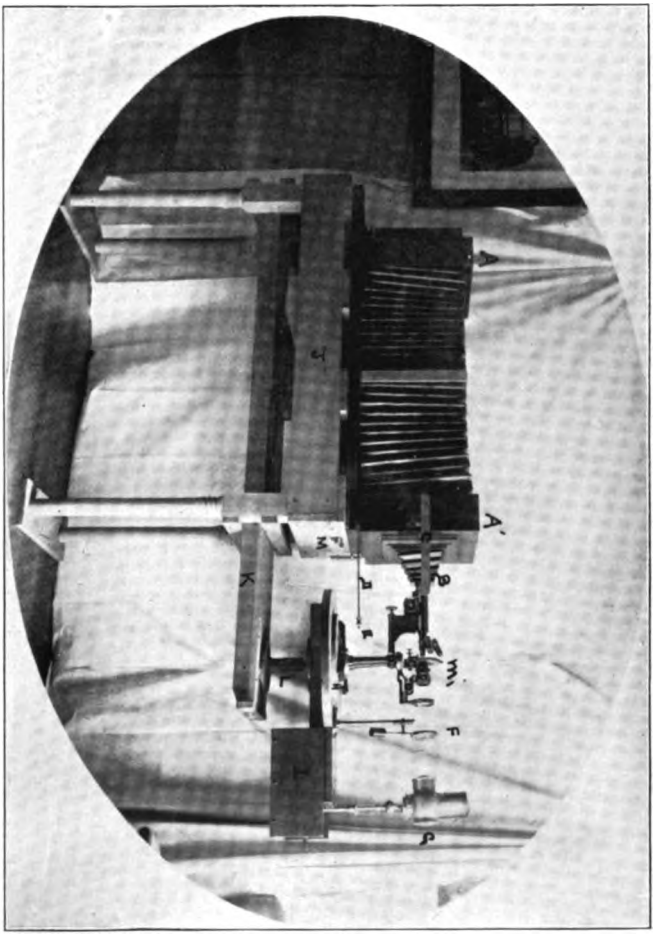

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PLATE II.

Showing apparatus in position for centering and adjusting object, arranging illumination, etc.

n.—set screw to fasten turn-table in position.

o.—scale extending full length of camera bed to show distance of photographic plate from object.

p.—iris diaphragm in microscope tube, just below the eye-piece, to cut off reflection.

Other lettering same as in Plate I.

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PLATE II.

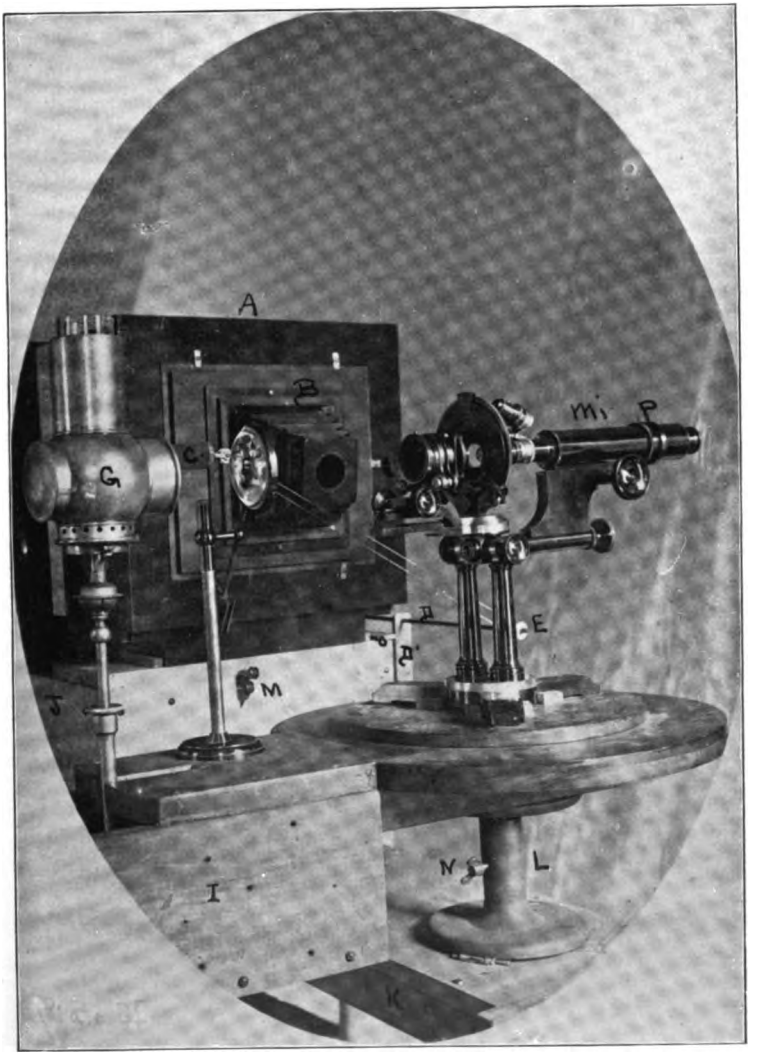

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PLATE III.

Showing apparatus ready for final focusing on screen of camera.

r.—pulley over which passes upper loop of fine adjustment string.

s.—scale on arm of sub-stage condenser, to show distance of sub-stage condenser from the object.

Other lettering same as in Plate I. and II.

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PLATE III.

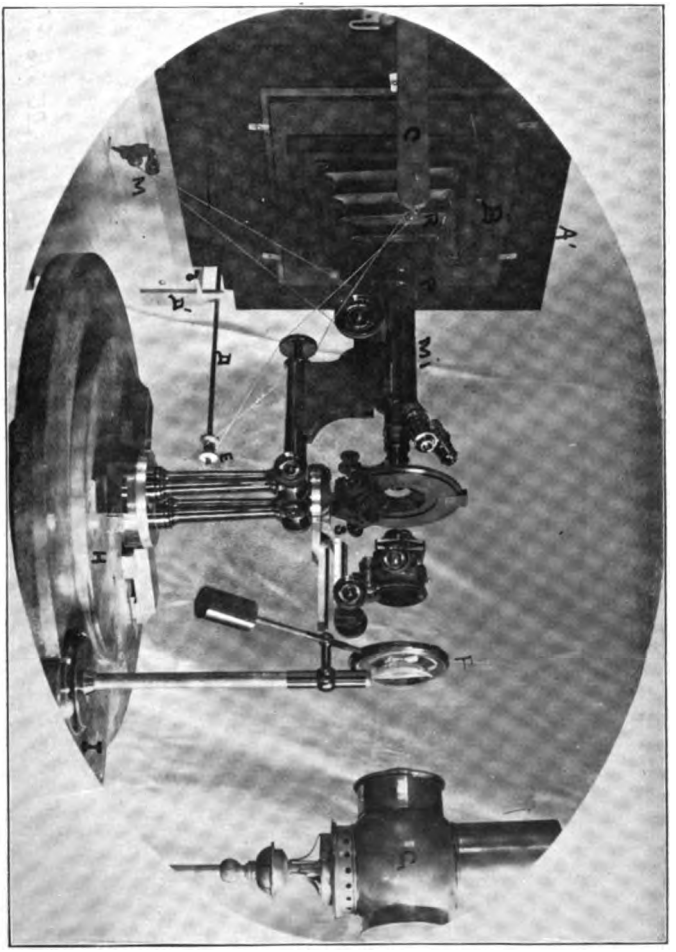

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PLATE IV.

Showing Bausch & Lomb Optical Co.'s professional stand with iris diaphragm, *a*, just below the eye-piece; and scale, *b*, on arm of sub-stage condenser, to indicate distance of substage condenser from object.

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PLATE IV.

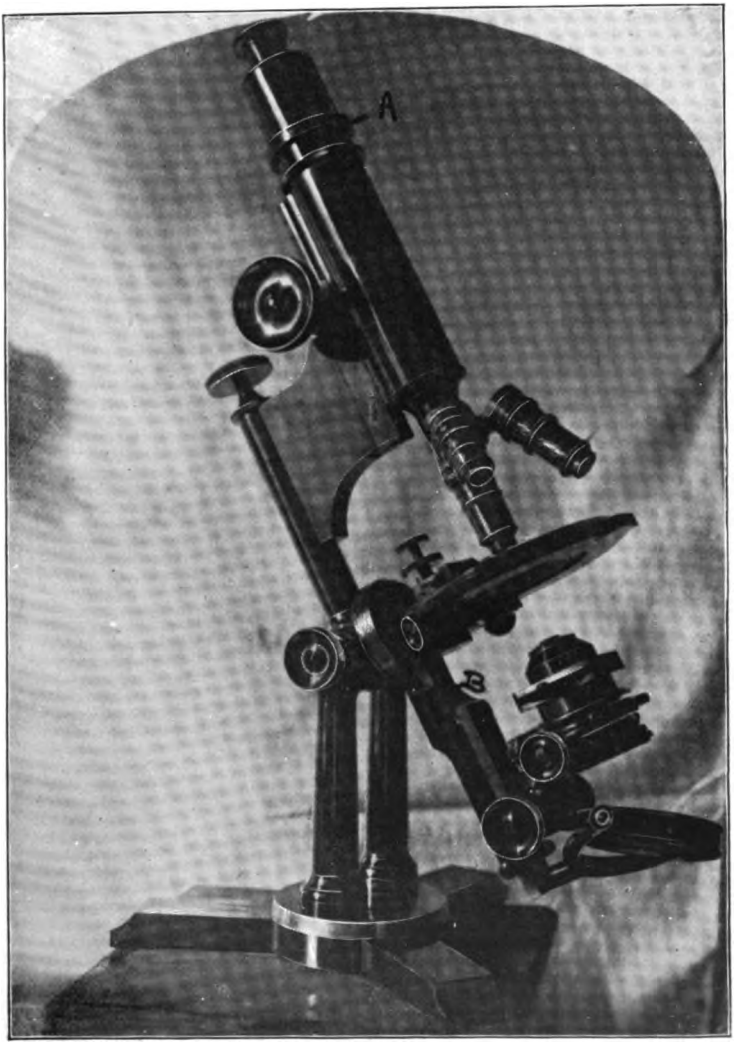

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PLATE V.

Showing apparatus for photographing objects in liquid, or any object that will not bear being placed in a horizontal position.

a. =set screw regulating height of rod, *e*.

a'.=set screw regulating height of plate holder.

b. =rubber band fastening black velvet sleeve to microscope tube.

c. =upright hollow tubing screwed into iron base, *f*.

e. =iron rod supporting plate holder and adjustable at various heights by means of set screw, *a*.

f. =cast iron base supporting microscope and apparatus.

o. =black velvet light-tight sleeve attached at upper end to frame carrying plate holder and at lower end around the microscope tube.

p. =removable plate holder.

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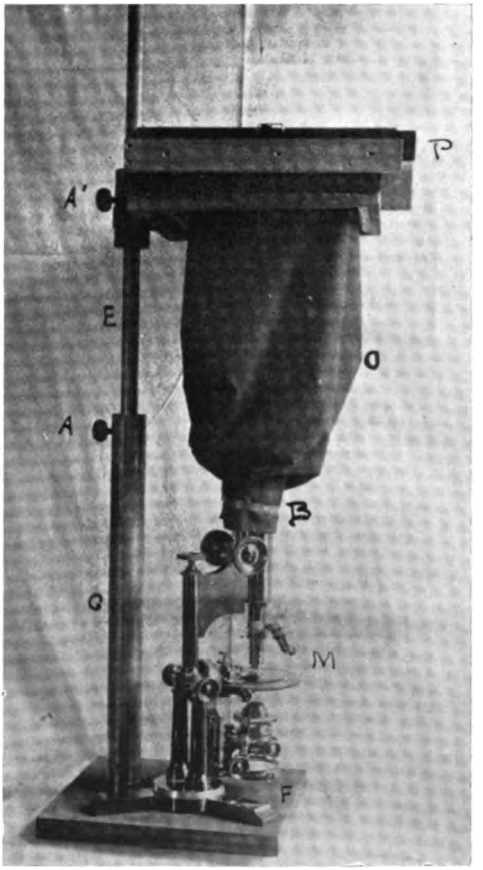
PLATE V.



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PHOTOMICROGRAPH VERSUS MICROPHOTOGRAPH.

(*Second Note*)

A. CLIFFORD MERCER, M. D., F. R. M. S., SYRACUSE, N. Y.

In addition to the first note under the above heading, published in the PROCEEDINGS of 1886, at page 131, I would like to add: The word Photomicrograph was first used in 1858. An account of its origin can be found in the *Liverpool and Manchester Photographic Journal* (now *British Journal of Photography*), August 15, 1858, at page 203, and in the same volume at page 414, and also in *Sutton's Photographic Notes*, Vol. III., at pages 205 and 208. In the latter, at page 205, J. T. Taylor writes: "By the way, will Mr. Shadbolt (who is an authority in microscopy), or anyone else, kindly suggest a suitable name for such magnified pictures? 'Microphotograph' wont do, because the name has been given to reduced pictures." At page 208, George Shadbolt answers: "The word microphotograph originated, I believe, with myself, and is applied, I think correctly, to very *small photographs*, not to photographs of *small objects*, which would more correctly be photomicrographs; but probably a convenient word for this class of subjects, as well as enlarged copies generally, would be megaphotographs."

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ASTRONOMICAL PHOTOGRAPHY WITH PHOTOMICROGRAPHIC APPARATUS.

A. CLIFFORD MERCER, M. D., F. R. M. S., SYRACUSE, N. Y.

On the twentieth of October, 1892, occurred a partial eclipse of the sun, which would have been visible in Syracuse, N. Y., had not clouds interfered. Early in the day, notwithstanding the overcast sky, I placed my heliostat on a shelf outside a south window*. Within the room I arranged a portrait lens of eight inches focus and a microscope in the same axial line**. The substage condenser was removed and a camera connected with the eye end of the microscope tube. Such sunlight as fell on the mirror of the heliostat was reflected through the portrait lens. The portrait lens projected an image of the clouded sun's disc, about one-twelfth of an inch in diameter, in the plane usually occupied by an object on the stage of the microscope. This tiny image was itself projected by a microscope objective of an inch and a half focus to form a second image, two inches and three-eighths in diameter, on the ground-glass of the camera. The clouds made sharp focusing impossible. Only an imperfect focus was obtained. The clock of the heliostat kept the image steadily on the ground-glass.

During the eclipse sensitised plates were substituted for the ground-glass. Exposures were made when the clouds were thin enough to permit. Thus six negatives were secured. Prints from two of these are submitted with this paper. In looking at these prints one is to imagine he looks to the south with east to his left, west to his right, north overhead and south toward the horizon. In the prints

* Fig. 39, page 147, Transactions American Microscopical Society, Vol. XIV., 1892.

** Fig. 38, page 147, Transactions American Microscopical Society, Vol. XIV., 1892.

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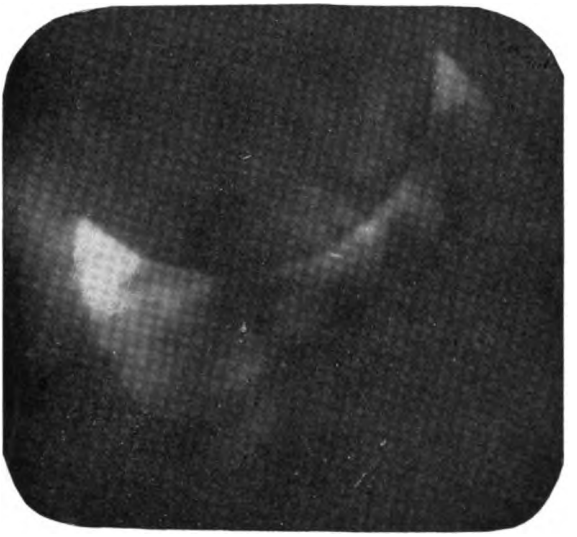

FIG. I.

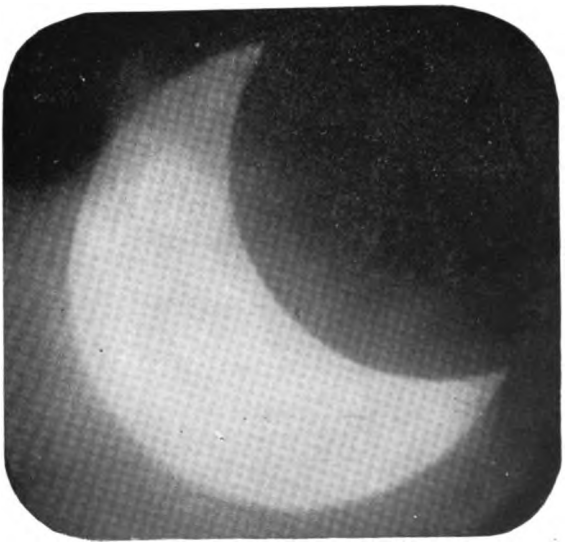

FIG. II.

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the relative positions of the discs of the moon and sun are reversed from right to left, from west to east. So the first print (Plate, Fig. I.) shows the moon's black disc advancing apparently from the north-east across the sun's disc, while the second (Plate, Fig. II.) shows the moon's disc passing off to the west.

This is, I believe, the first record of an attempt to use photomicrography astronomically. All the necessary apparatus could be easily packed in a trunk, possibly in a hand-box. Such apparatus is suitable for long distance transportation. If an unaided telescope objective were used to project an image of the size obtained in our negatives, a focus of twenty-one feet would be required; and the lens would have a diameter of about sixteen inches, if made as such lenses are usually. Such an objective properly mounted would result in an instrument nearly half as large as the great Lick telescope, with its photographic objective. In other words, by using a portrait lens having a focus of fifteen or sixteen inches, a size commonly used for "cabinets" in photographers' studios, instead of the portrait lens I used, the apparatus I have described would produce a negative image equal in size to that produced by the unaided Lick lens; or, leaving my portrait lens in place, the same result could be obtained by substituting for the microscope objective of one inch and half focus another of about double the power, one of three-quarters of an inch focus. The Lick instrument has a tube about fifty feet long and forty-two inches in diameter, while my apparatus has two tubes less than one foot long and about one inch and six inches in diameter respectively. To the smaller tube is attached a camera with a bellows extending from one to six feet. Stability and freedom from vibration are comparatively very easily obtained with the small and short apparatus. The difference in housing room is great. The difference in cost is enormous. It is evident that in several respects the photomicrographic arrangement has advantages over the great Lick photographic instrument.

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If, however, we turn to the matters of light and separating power, the very great superiority of the Lick objective is seen. The results given in the following tabular comparison are only approximately accurate, and I have not taken into account the loss light suffers by absorption as it passes through glass and by reflection at incident surfaces, the Lick objective consisting of three thick lenses and the photomicrographic arrangement having more than twice as many, but comparatively very thin, lenses and the mirror's reflecting surface:

	Lick photographic objective.	Larger portrait lens.	Smaller portrait lens.
Diameter of objective	33 in.	3.75 in.	2 in.
Focus of objective	550 in.	15 in.	8 in.
Focus divided by diameter	16.66	4	4
Relative value of light in first image	1	16	16
Size of first image	5.1 in.	.1395 in.	.0744 in.
Total equivalent focus, 550 inches, divided by diameter	16.66	147	275
Relative value of light in final image	1	$\frac{1}{77}$	$\frac{1}{277}$
Time of exposure, eclipse of sun (about)	$\frac{1}{1000}$ sec.	$\frac{1}{10}$ sec.	$\frac{1}{2}$ sec.
Separating power	1	$\frac{1}{8.8}$	$\frac{1}{18.88}$

The tabular comparison needs no explanation, I think, excepting, perhaps, the meaning of "separating power." Other things being equal, separating power varies with the aperture or diameter of the objective. If the Lick objective, having an aperture of thirty-three inches, could barely show a certain double star as two distinct stars, it would be impossible for any objective having an aperture of four or two inches to show such a double star as two distinct stars. A star apparently single when seen through any objective having an aperture of two inches might be seen to consist of sixteen or seventeen stars in line, almost touching one another when seen through the Lick photographic objective. A star apparently single when seen through any objective having an aperture of three inches and three-quarters might be seen to consist of eight or nine stars in line, almost touching one another, when seen through the Lick photographic

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objective. This power of resolving an apparent single star into two or more, or of showing the details of sun spots or other objects, is known as separating power. Notwithstanding the tabular showing, the photomicrographic arrangement has advantages in some respects as already mentioned; and to these should be added the superior correction of aberrations now possible in lenses made of small discs of glass which are produced in great variety as to optical properties, a variety not yet realised, I believe, in large discs.

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ACETYLENE GAS AS THE ILLUMINANT IN PHOTOMICROGRAPHY.

W. H. WALMSLEY, CHICAGO, ILL.

In a paper on Photomicrography, read at the meeting of this society at Ithaca, in 1895, I stated that the lately discovered acetylene light bade fair to be the best of all artificial illuminants for this work. At that time, my knowledge of it was limited to the occasional and ofttimes incorrect accounts of its wonders furnished by the newspaper press. I had never seen its light, and practically was utterly ignorant upon the subject. But the descriptions of its extraordinary brilliancy, whiteness and steadiness, seemed to infer a high photographic value, and led me to predict what has since that recent date been abundantly proven to be absolutely correct. To be sure, an English scientific (?) journal about that time asserted that exhaustive experiments showed the light to possess no photographic value whatever, and that the expectations of those who proposed to use it for the purpose were doomed to disappointment. It is not the first recorded instance of expert mistakes. Facts are stubborn things, and now, when acetylene is being used not only in photomicrographic work, but for making exposures in the daily work of photograph galleries, it must be conceded that the journal in question was mistaken in its statement.

A perfect light has been the desideratum of the worker in photomicrography from the beginning. Direct sunlight properly controlled and directed is very nearly but not quite this. It varies greatly in intensity with the season and the hour of day. It is rarely available in the confined limits of a city work-room and never at night, the only time when most of us have leisure to devote to this work. A heliostat and

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other more or less costly apparatus are necessary for use with it. None but the favored few can ever hope to invoke its aid with any degree of regularity.

Next in order of perfection, the oxy-hydrogen or lime light may possibly be placed, though acetylene is certainly its worthy peer. Provided with a pair of charged cylinders, a properly constructed jet, and skill in the manipulation of the apparatus, the photomicrographer has at his command a very perfect and entirely satisfactory source of light for his work. But the necessarily considerable cost of the outfit with the expense of maintaining it, to say nothing of the difficulty or impossibility of recharging the cylinders, in many localities, must always restrict its use to a very limited field.

The light of burning magnesium is exceedingly actinic and useful for photomicrography, permitting very short exposures. But numerous objections have always prevented its extensive use. The fumes and heavy volumes of smoke attendant upon its combustion are very unpleasant, to put it mildly. It is difficult to determine the proper length of exposures, and the cost is somewhat of a serious item to many. The electric arc light is most excellent, but, for obvious reasons, quite beyond the reach of most of us.

It follows, then, that to the present time, the one and only generally available light for our work has been that of the old, familiar, omnipresent coal-oil, kerosene or petroleum lamp. Far be it from me to say aught against an old friend upon whose services I have relied for many years. Rather let me give thanks for the good it has done. Yet it must be confessed its many faults fully equal its virtues. Its perfume is not that of roses nor its temperature suggestive of coolness on a hot summer evening. Its yellow, non-actinic rays, though suitable for some classes of subjects, are quite the opposite with a majority; requiring prolonged exposures, tending to a slight fogging of the plate, with increased danger of a bleared image from movement of the camera or microscope. Its negatives rarely possess the brilliancy or crispness of those made by whiter and more actinic lights.

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I have made more than a thousand negatives with its aid, but very few of them compare favorably in these particulars with those I have exposed under the acetylene light during the past eight or ten months.

Early in the autumn of last year it was my good fortune to make a practical acquaintance with acetylene, under circumstances and conditions which speedily led to familiarity, and it has continued to be a daily and nightly companion ever since, with increasing appreciation of its superlative value for all purposes requiring artificial illumination. Absolute steadiness, light brilliant and white, but utterly devoid the ghastly whiteness of the Welsbach burner, in fact "a chunk of sunlight" (the spectra of the two being almost identical), no appreciable heat, no odor whilst burning, moderate cost of the apparatus for generating it, with extreme portability, simplicity, safety and ease of manipulation, and almost inappreciable expense of maintenance, combine to render acetylene the ideal illuminant in photomicrography.

It is not my intention in this paper to say aught regarding the manipulation of light, microscope or camera, leaving those subjects to other and abler pens. Our respected president, Dr. Mercer, has already published such exhaustive, yet simple, and admirable articles on these subjects that nothing original remains for me. I should merely quote from him were I to enter upon them. I shall, therefore, speak only of the light itself. This is so highly actinic that it may be safely stated, in a general way, that the average length of exposures are not over one-fifth of those required with the best coal-oil lamp, in many cases not exceeding one-tenth of the latter.

The form and size of burner may be varied at will, though it should never exceed one cubic foot per hour in capacity. My most satisfactory work has been done with burners of one-tenth that size. To insure perfect steadiness, it should be enclosed in a metallic case with glass front, as the acetylene flame is very susceptible to draughts of wind. I have here four different arrangements of burners and cases,

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all of which have proved extremely satisfactory. The first is a Russian iron box, 4 by 4 by 2 inches, with open top and bottom, the front of glass and the whole carried on an upright rod, to which it can be secured at any desired height above the table. The burner is of one-foot capacity, set edgewise to the glass front, affording a very brilliant and powerful light. The second consists of three pencil-like flames set in line one behind another, each having a metal diaphragm in front with an opening of one centimeter. These being set in one line, an intensely brilliant light of great depth is obtained with a very small consumption of gas. For the general idea embraced in this arrangement I am indebted to Dr. G. M. Sternberg, Surgeon-General, U. S. A., who used it a number of years ago with ordinary street gas, and described it in his well-known and valuable work on Photomicrography.

The third arrangement is simply one which I originally designed for a microscopic illuminator, but which has proven in practice to be still more valuable for photomicrography. It consists of a burner of about one-eighth cubic foot capacity, set in a small metallic casing, with front closed by an ordinary glass microscope slide, which can be removed or replaced at a moment's notice, if broken. The glass may be dispensed with if desired. Its only use is to guard against possible draughts and to insure absolute steadiness in the flame. The interior of the case may be whitened by plaster-of-Paris or coated a dead black, at will. I prefer the latter. This little lamp is carried upon an upright, with adjustments for height and inclination. The light is exceedingly brilliant, absolutely white and the consumption of gas almost infinitesimal. A modification of this lamp, suggested by Hon. A. A. Adee, of Washington, is also shown. In this, the glass slip front is replaced by a blackened strip of brass, with an opening of one centimeter opposite center of the flame. It is very satisfactory in use, but I have not found it to be any better than the plain glass slip.

My own practice is to render the light monochromatic by

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means of a cobalt blue cell placed in substage of microscope. If both focusing and exposure be made under this light, I think there can be no doubt of superiority in sharpness of definition and crispness in general effect of the negative. The length of exposure is somewhat increased, but not to any great extent.

Among the many advantages of acetylene not the least is the absence of appreciable heat attending its use for our purpose. No one who has attempted to make a photomicrograph by the light of a coal-oil lamp on a warm night has failed to be sensibly impressed with its fervid qualities; a good friend indeed, but rather too warm for the season. The small area of the acetylene flame and limited consumption of gas render it an essentially cool one, and entirely odorless as well.

The crowning advantage of acetylene, however, is its absolute uniformity. Sunlight varies under differing conditions, as is well known. So do all artificial illuminants hitherto in use. The so-called standard candle power is a delusion and a snare. It has been tolerated so long as the unit in light measurements simply because nothing better has hitherto been available. But it seems to me that in acetylene we have the long hoped for and perfect standard. This gas seems to be staple ($92\frac{1}{2}$ parts of carbon to $7\frac{1}{2}$ parts of pure hydrogen), whether generated from calcium carbide, yielding four cubic feet or less to the pound, or the very richest, of more than six cubic feet. Given a burner, a known capacity, say one consuming a cubic foot per hour under a predetermined and uniform pressure, for instance, of two inches of water, the light produced would always be the same, no matter whence or by whom. It would thus seem to be a very simple matter to fix upon a burner of suitable capacity and the best pressure to give to the world a standard unit of light, from which measurements could be made with a far greater degree of accuracy than is possible at present. A two-inch pressure is suggested, as being about that used in the distribution of ordinary illuminating gas. It might be also that a burner of much less capacity than one foot would be better than the

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latter. Careful experimentation by competent persons would doubtless give certain and reliable results. I am only able to make the suggestion. I think it is a subject entirely within the scope of our society, and earnestly recommend that it take some action in the matter. It would be a very gratifying thing for it to have the honor of naming a universal standard unit of light. In my opinion, this is to be found in acetylene. Will not our society take hold of the matter in earnest and prompt manner?

An important factor in the successful use of acetylene for photomicrographic as well as other purposes, is the steady, automatic generation of the gas from calcium carbide, and its delivery, under uniform pressure of proper extent, to the burners in a dry, cool state. These conditions appear to be very satisfactorily filled by the "Monitor" automatic acetylene generators, manufactured by my house, Walmsley, Fuller & Co., of Chicago. These machines are extremely simple in design and construction, easily managed by anyone, perfectly automatic in operation, and absolutely safe. A nominal water pressure, never exceeding three inches, is all that can possibly be used with them. The holder carrying the carbide serves also as a gasometer, rising in the tank and lifting the carbide out of the water, so that generation of gas ceases when not burning. They are made in many sizes, all however embodying the same general principles. The gas may be turned on or off at pleasure and is always ready for use at a moment's notice. The smallest size will light one or two reading lamps, the larger a house or a block. They are moderate in cost, both as to that of the machines themselves and the production of the gas. At the present price of calcic carbide the cost of the light, compared with a given amount obtainable from ordinary street gas, is about the same as the latter would be at eighty cents per thousand feet, one cubic foot of acetylene giving as much light as thirty feet of ordinary gas.

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ZOOPHILY VERSUS HOMOPHILY.

PIERRE A. FISH, D. Sc., Cornell University, Ithaca, N. Y.

The onward march of events, accompanied by new conditions and new methods, has given a much wider significance to the term vivisection than was formerly attached to it. It is quite commonly regarded, by those opposed to the practice, as a method of inflicting, by dissection upon a living conscious animal, excruciating pain for the gratification of the operator's curiosity, or for the exhibition of some experiment already demonstrated and thoroughly familiar.

Technically a man or animal is vivisected when a hypodermic needle is thrust into the skin for the purpose of alleviating the pangs of disease by this form of medication ; while if any part of the body be crushed or mangled by a blow, as from a club, resulting in serious injury, there is infinitely more suffering but not literally vivisection, since the tissues are not cut but bruised. Nor is the condition of the victim taken into account ; a serious and prolonged operation upon an anesthetised animal may result in as little discomfort to it as the blows or accidental injuries received by any dog on the streets as an incident in his career ; or, in the former case, if the operation result in death, the end is without pain.

But the issue that now seems to be nearest the surface, under this really comprehensive term of vivisection, is whether it is justifiable to utilise, even to their destruction, animals for the real or probable benefit of mankind, and with this as the issue, there would be the natural classification of those who, influenced by genuine sentiments of mercy and a desire for the alleviation of suffering, band themselves together for its suppression, especially in animals, on account of the inability of the dumb beasts to make known certain existing

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abuses and because of their helplessness in competition with men. This class of persons would encompass the animals with so many and rigid safeguards that if really put into practice many lines of progress would be materially restricted. For our present purpose let such persons be classified as Zoophiles—antivivisectionists—and the vivisectionists as Homophiles, the latter advocating not only the essential doctrines of the former but something more. Actuated by the same sentiments of mercy and regard for suffering they would, when obliged to inflict pain for the general good, minimise it to the greatest possible extent, by the use of anesthetics or otherwise.

The practice of vivisection, like the theory of evolution, does not appeal to the finer esthetic qualities of mankind ; it is not intended to ; it would appeal rather by an array of self-evident facts to the importance and necessary usefulness of the practice.

Is it justifiable to sacrifice an animal from a lower level in the zoological scale for the preservation or benefit of another animal in that same scale ? Nature has already decided that question by the creation and maintenance of the order of carnivorous animals, which live on flesh alone, and others which subsist on mixed diets. If the zoophiles understood and were enabled to trace the preparation of their animal food from the living creature to the time it is ready for eating, would they still exercise as much pertinacity in their denial of the right to use lower forms for the benefit of the higher, or is Nature again at fault in fashioning the human digestive apparatus so that a mixed diet may be enjoyed ? Would not a logical adherence to their cardinal principles preclude anything but a vegetable diet, and extend even to the matter of clothing and personal adornment ?

But the answer is made that it is not the aim to legislate against animal inoculations for the determination of disease, but to supervise and to eliminate the promiscuous and unnecessary use of it. Much stress is also laid upon the tortures which have been inflicted upon animals in the past, and these remote

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instances, although exceptional when the vast number of experiments are considered, are resurrected and represented as being in common usage at the present day. There is no practice that may not be abused. Are the principles of the Christian religion, upon which so large a portion of the civilised world depends for encouragement and support during the battle of life, to be undermined because long years ago there were certain enthusiasts whose zeal permitted them to inflict the most excruciating, cruel and unparalleled tortures upon their fellow-beings "In His Name"? Is the future saving of human life, the saving of vast sums of money by the preservation of the lives and eradication of disease in domesticated animals, and the search for the truth which elevates to a higher plane of civilisation to be retarded by the misdeeds of past offenders? Will statistics confirm the generalised statement that vivisection tends to brutalise the operator? Such an accusation is not brought against a surgeon in the performance of his duties. Wherein lies the great difference in the degree of vivisection? How many of the antivivisectionists have really gone beyond the first shudder at the thought of the existence of pain and appreciated the fact that life itself is a struggle for existence, and that the perception of a greater or less amount of pain, under ordinary conditions, is a circumstance in the career of every living creature?

The vivisecting experiments of Galvani have illumined not only the scientific world, but the material world as well. Out of the crude apparatus of a vivisector have been developed the wonderful electrical appliances of today. Galvani's experiments were the keys which unlocked the doors of ignorance, not only as to certain physiological phenomena, but the manifold mysteries of the uses of electricity, many of which are still unsolved.

The fact that mature and deliberate judgment may be exercised in a question of such vital interest has been recently exemplified in one of the Cantons of Switzerland, where a measure for the total prohibition of vivisection was submitted

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to the population *ad referendum*, with the result that 40,000 votes were cast against such prohibition and only 17,000 for it.

In the District of Columbia, it has been proposed to legislate against vivisection, or, at least, to regulate it by the maintenance of certain inspectors, who shall at intervals visit the laboratories or other places where the practice is carried on.

The bill as arranged is unnecessary, unreasonable, retrogressive and reactionary in its tendency.

It is unnecessary, because the great majority of vivisectors are intelligent, earnest and humane gentlemen, whose object in animal experimentation is to suppress and prevent the occurrence of disease, or to add some new fact for the welfare of mankind.

It is unreasonable, in that it advocates a system of espionage in which the inspector may be a person ignorant, unskilful and unappreciative of the object to be investigated or of the methods employed therein. It is manifestly unfair to permit such a person to officiate as censor, and is a malicious insinuation against the integrity of the investigator.

It is retrogressive, because it prevents further research; medical and biological sciences can progress only through experimentation.

It is reactionary, because in the effort to encompass the animals with so many safeguards their use for the real benefit of mankind is lost sight of, and one should be reluctant to assume that the antivivisectionists love animals more and their fellow-beings less.

Pain is an adjunct of life, and its merciful infliction upon lower forms is not only justifiable but necessary when it may alleviate human suffering. Humanity is above animality and as long as Nature endows living animals with sensitive tissues, just so long will pain exist.

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ON THE TEETH OF MAZODUS.

E. W. CLAYPOLE, B. A., D. Sc., F. G. S, Akron, Ohio.

On the top of the Berea grit, as it occurs at Berea, lie about twenty feet of very dark shale, in which occurs a great profusion of certain fossils, especially *Lingula melie* and *Discina Newberryi*. With these are some fish remains, and in the lowest portion are the large, flat, pavement teeth which were named *Mazodus* by Dr. Newberry in his Paleozoic Fishes of North America. They evidently formed the mouth armor of some paleozoic shark of comparatively large size, some of the teeth exceeding two inches in diameter.

The great size and good condition of these teeth, which are all that we yet know with certainty of the fish, render them good material for microscopical investigation, and I propose in this note to give the results of my examination so far as it has yet gone.

The vertical section of the tooth shows a loose mineral structure permeated by canals, presenting the same appearance as those in bone. They run, on the whole, vertically through the tooth, but branch and anastomose with such frequency that the whole structure is penetrated or honeycombed with them. Radiating obliquely from these into the solid tissue are numerous canaliculi running in directions determined by that of the section and branching as they diverge from the canal and from each other.

No lacunæ are present and if the canaliculi anastomose they unite with those coming from other canals—a structure quite common in the teeth of fishes.

In the lower portion of the tooth the direction of the canals is so irregular that little appearance of parallelism can

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be detected. The base has a loose spongy texture, gradually becoming more firm and regular upwards.

Traces can be seen in the lower part of the tooth of an irregular laminated structure in the solid matter, like that in the Haversian system of bone, but toward the summit the structure becomes much more regular and the somewhat wavy striation of dentine is evident. Through this dentine, however, the canals extend with nearly the same frequency as below, and the radiating canaliculi accompany them everywhere. This portion of the tooth has been less deeply stained during mineralisation, and thereby betrays a denser nature than that of the lower portion.

Mazodus, therefore, presents a structure very like that of *Cladodus*, which I described in a paper before this society at Madison, in 1893. It shows what we may call a dentary tissue, which is not the differentiated tissue composing the teeth of the higher animals, and which does not show even a well marked dentine, though an approximation to that form of tissue is discernible. But the passage of the canals through it makes its appearance very different from that of typical dentine.

It is further to be noted that as the canals run completely through this outer layer they open on the external surface of the tooth, where their mouths give a pitted appearance to the fossil, often visible without a lens. It is not easy to correlate them, therefore, with Haversian canals, which are always lined with periosteal membrane and never detail upon an external surface. What purpose was accomplished by their presence in the superficial layer it is not easy to see. The teeth seldom show signs of great use, though their purpose was probably the crushing of shells, a task likely to wear or even to break them. Deep scratches, however, are not uncommon. They were not, probably, renewable after loss or injury, as are the cutting teeth of the sharks, and hence their loose and spongy structure is the more remarkable.

The teeth present no trace of enamel, nor have I found any substance deserving the name on any paleozoic tooth

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that I have examined. Should this be generally true, then the introduction of the hardest material in the animal body is of comparatively late date. At the same time, its absence would indicate some difference in the development of the teeth, for the enamel is a product of the ectodermic layer, while the dentine and its analogue are of mesodermic origin.

It would then follow that the involution of the ectoderm by the development of the enamel organ did not take place among the early sharks.

The literature within my reach does not enable me to ascertain how far investigation has proceeded into the history of the evolution of the teeth in existing sharks. This matter has received much attention among the higher animals, but our knowledge of dental evolution and development in fishes is as yet very incomplete.

The facts here given show that Dr. Newberry's original description needs a slight correction if his words are interpreted in their strict sense. He says: "They (the teeth of *Mazodus*) are composed of dense enamel-like tissue throughout, with no division into crown and base, one enamel and the other bone, such as we find in all known crushing teeth of elasmobranch fishes." So far from being "enamel-like," save perhaps superficially to the naked eye, these teeth are composed of a very spongy dentinal tissue, whose traversing canals reach to the very surface, and which, therefore, differs radically from the close, fine, crystalline structure of real enamel. And further, so far from being an exception to the rule among elasmobranch fishes, they are in full accord with it. In whatever cases I have examined the teeth of fossil sharks I have found them uniformly composed of this loose, unenamelline tissue and a true enamel conspicuously absent. I need scarcely add that this remark refers to paleozoic times only.

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FIG. I.

Mazodus tooth.

Transverse section, showing canals and canaliculi.

FIG. II.

Mazodus tooth.

Longitudinal section, showing dentine and canals.

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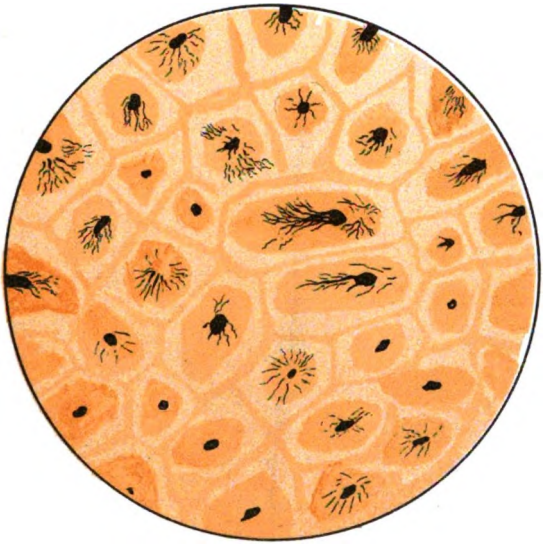

Fig 1.

Fig 2.

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ON THE STRUCTURE OF SOME PALEOZOIC SPINES FROM OHIO.

E. W. CLAYPOLE, B. A., D. Sc., F. G. S., AKRON, OHIO.

In the TRANSACTIONS of the American Microscopical Society for 1893, I gave some account of the structure of the teeth of the cladodont sharks of Ohio, as found by Dr. Clark in the Cleveland shale of Cuyahoga county. In the present paper I will give a few details of the structure of some spines and teeth from the same field, but from a rather higher horizon, the lower carboniferous.

Overlying the Cleveland shale in which the former species were found is the Berea grit, the lowest of the sandstones of the carboniferous system in Ohio. It is not usually rich in fossils, but its topmost layer at Berea is a solid bed of iron pyrites, about two inches thick, and scattered over this, or imbedded in it, lie numerous spines of different kinds, the most abundant of which is one that was described by Dr. Newberry, in his work on the Paleozoic Fishes of North America, as *Ctenacanthus angustus*. These spines are, of course, in a somewhat imperfect condition, owing to the nature of the preserving medium, but the characters are recognised with little difficulty. It is not improbable that more than one species of *Ctenacanthus* may be present, as species are at present defined.

The spine examined is rather long and slender, gracefully curved, laterally flattened and denticulated along its front edge. In all probability it formed the foremost ray of the dorsal fin of one of the sharks of that time. It is deeply furrowed at the back near the base and was set in the flesh without bony attachment, just as in the spiny sharks, such as the picked dog-fish of the present day. A transverse

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fracture shows that the hollow at the base is continued up through the spine as a tubular cavity, which simulates the medullary cavity of bone, but has no true analogy with it. This cavity is, of course, now filled with pyrites, and shows dead-black in the field of the microscope. The hard tissue surrounding this medial cavity is homogeneous and penetrated in all parts by large and numerous Haversian (?) canals. These run, in the main, parallel with the length of the spine, but they inosculate continually so as to form a complete network of minute passages, which are also clogged with pyrites and show in strong contrast against the reddish ground of the tissue. So numerous and large are these channels that in many places the empty spaces exceed the solid matter of the spine, which must consequently have been very light in comparison with its size, and therefore better adapted to its duty.

The ground mass of the bone is perfectly solid, without lacunæ or canaliculi, and shows in this point no resemblance to true bone. Traces of a concentric arrangement around the canals (in what may be called the Haversian systems), indicating the method of formation, may, however, be detected.

The structure, evidently, is more like that of tooth than of bone. This is what might be expected, as the spine must be classed with the dermal appendages rather than with the skeleton. The teeth, spines and scutes or scales that more or less cover the bodies of fishes are all special developments of the papillæ of the skin, and though in some kinds of teeth lacunæ are apparently present it is more than doubtful if they are of the same nature as the true lacunæ of bone, except when they are found in the cementum, which is merely the calcified periosteum of the alveolus.

The occurrence of *Ctenacanthus angustus* in the same horizon with *Mazodus*, and the absence of other forms, leads the geologist to suspect that they belonged to the same fish, and in that case one of the names will become a synonym. This, however, belongs to the field of paleontology.

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FIG. I.

Spine of *Ctenacanthus angustus—Nby.*

Transverse section, showing Haversian canals.

FIG. II.

Spine of *Ctenacanthus angustus—Nby.*

Longitudinal section, showing central cavity and canals.

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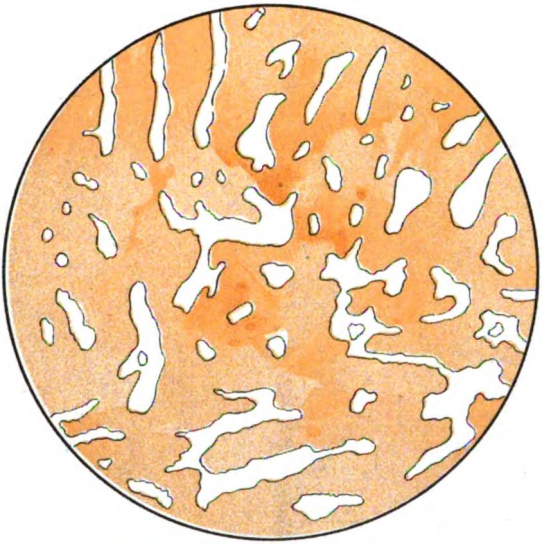

Fig. 1.

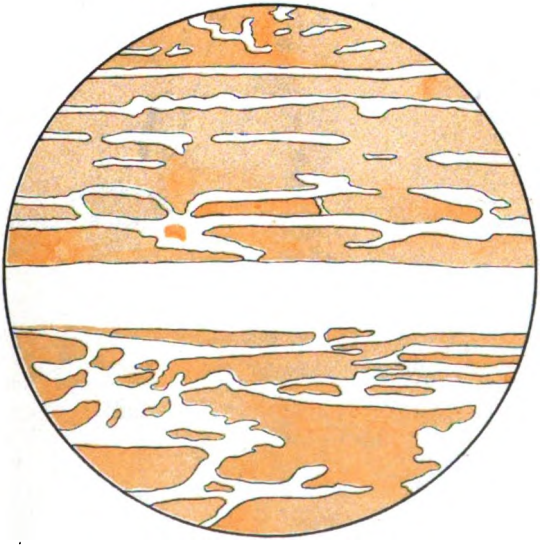

Fig. 2.

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THE ROTIFERA OF SANDUSKY BAY.

D. S. KELLICOTT, PH. D., F. R. M. S., COLUMBUS, O.

I.

In order that the title and the substance of my paper may not appear to be out of all reasonable proportion, permit me an introductory word of explanation. In consequence of the liberality of the Trustees of the Ohio State University, I now have a Lake Laboratory, located on Sandusky Bay, where I have spent a part of the present summer vacation and where I expect to spend more or less of subsequent ones. The study of the Rotifera of the bay will be continued from year to year, and it is my purpose to report from time to time the work accomplished. This list, then, is only a partial one, giving such species as have been identified, their habitats, etc., with descriptions of such as appear to be new.

While living at Buffalo it was my pleasure to study Rotifera somewhat at the eastern end of Lake Erie. Very few of my notes have been published, but the resumption of study at Sandusky renews acquaintance with many old friends, which it has been a delight to meet and greet. This charming group is one of deep interest everywhere, but in no situation may one expect to find a richer rotiferal fauna or one with more unique forms than in our Great Lakes.

Since the brief papers read by myself on these creatures at the Cleveland and first Pittsburg meetings some work has been done and published on Rotifera of the Great Lakes, notably by Mr. H. S. Jennings. His paper, A List of the Rotatoria of the Great Lakes and Some of the Inland Lakes of Michigan, will often be referred to by the name of the author in the course of my remarks.

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Sandusky is situated on a peninsula, having the wide glacial estuary of the Sandusky river, known as the West Bay, on one side, and on the other the East Bay, which is a shallow body of water cut off from the lake by a sandbar, known as Cedar Point, several miles long. This East Bay is the site where the work of this paper was done. The bay extends to the southeast several miles, ending in extensive marshes, where a variety of marsh plants abound in great profusion. Moreover, there are sheltered ponds and marshes on the Point, which are simply bodies of water resting in basins of sand, maintained at a constant level by the influence of the lake. The place appears, therefore, to be an ideal home of Rotatoria, and the short time thus far spent in finding them out has not been disappointing.

The systematic arrangement followed is that of Hudson and Gosse's Rotifera. References to this work will be simply by the authors' names.

FAMILY I. FLOSCULARIADÆ.

FLOSCULARIA, Oken.

1. *F. ornata*, Ehrenberg.

Very common on the dissected leaves of *Utricularia*, on the under side of leaves of *Nymphæa* and *Nelumbium*, in the bay and in the marshes and coves.

I often found the egg placed high in the tube, so that when the animal retreated it passed below the egg.

2. *F. cornuta*, Dobie.

Few of this distinct species were found; elsewhere, Buffalo and Central Michigan, I have found it as abundant as *ornata*.

3. *F. Millsii*, Kellicott.

In abundance in *Utricularia* growing in a marsh on Cedar Point. This makes the third station for this beautiful floscule, viz.: Black Creek, Ontario, Canada; Sidney, New South Wales, by Mr. Thomas Whitelegge; and Sandusky Bay, Ohio.

I have again measured an average example, and find sub-

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stantial agreement with the dimensions given in my original paper,* whilst that of the antipodal form was scarcely half as large. Again, in regard to the foot, I may say that there is no attenuated pedicle, but as Mr. Whitelegge says, "A short [and stout] immovable stalk, which is not affected when the animal retracts or extends;" the three ring-like protuberances at the end of the foot, just above the stalk, described by him, I am unable to make out.

4. *F. campanulata*, Dobie.

Exceedingly abundant in the quiet marshes, found on a variety of plants, but most numerous on young branches of *Utricularia.*

5. *F. ambigua*, Hudson.

With the preceding; much less numerous.

6. *F. mutabilis*, Bolton.

Few taken in the skimming net and by straining water from the faucet.

FAMILY II. MELICERTADÆ.

MELICERTA, Schrank.

7. *M. conifera*, Hudson.

Very abundant, especially under floating leaves of *Nelumbium.* Its tubes were often found in clusters similar to those of *M. ringens.* I have found groups of tubes fully one-eighth of an inch long and containing not less than twenty-five tubes.

8. *M. flocculosa*, n. s.

Lobes wide, expanding fully twice the greatest width of the stout body; antennæ very short; tube gelatinous, with much adherent floccose, thus resembling a dense tube of a floscule.

Two individuals of this fine species were found under floating leaves of *Nelumbium luteum.* It appears to be closely related to *M. tubicolaria.* It differs from that species in

* TRANSACTIONS of this Society, Cleveland meeting, 1885, page 48.

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having a much stouter body, the lobes relatively narrower, in having a spatulate chin and very short antennæ; *tubicolaria* has these organs very long. The tube appears to have the floccose incorporated with the gelatinous matter. This, I think, is done by accumulating it in the ciliated cup and placing it on the margin and surface of the tube, much as *Œcistes longicornis* may be seen to do.

One characteristic, large, brown egg was noticed in the tube of the largest one.

The size is large, but the record of its exact dimensions was lost.

LIMNIAS, Schrank.

9. *L. ceratophylli*, Schrank.

Very few were found. I have found this abundant in the Niagara River.

10. *L. Shiawasseensis*, Kellicott.

Very rare.

11. *L. annulatus*, Bailey.

Very common on aquatic plants in coves and marshes.

CEPHALOSIPHON, Ehrenberg.

12. *C. limnias*, Ehrenberg.

Abundant on *Utricularia* and water-lily pads.

ŒCISTES, Ehrenberg.

13. *Œ. crystallinus*, Ehrenberg.

Not uncommon.

14. *Œ. longicornis*, Davis.

Not uncommon.

I find that this interesting rotiferon, as I reported several years ago, has a well-formed cylindrical or somewhat vase-shaped tube, covered with floccose, arranged in transverse lines, giving the striated appearance alluded to by Mr. Davis. When the focus is deeper the tube looks like a piece of animal's hide with its long shaggy hair. This disposition of the material is doubtless accomplished by means of the stout

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ciliated chin. Mr. Jennings finds the tubes of definite form.

The corona appears to me to be more strongly bilobed than the extant figures represent it. The tube closes when the animal retires within, and as it cautiously returns, *one* antenna, partly folded over the front, precedes, much like the antenna of *C. limnias*, although without the exploring motion of that species; later the other long antenna unfold and extend its sensitive setæ.

15. *Œ. mucicola.* Kellicott.

Somewhat rare. The gelatinous balls of the alga in which it lives are numerous enough, and occasionally a rotiferon is seen protruding or suddenly retreating within its domicile for protection.

LACINULARIA, Schweigger.

16. *L. socialis*, Ehrenberg.

Very abundant on *Nymphæa* and *Nelumbium.* I have seen colonies a fourth inch across.

MEGALATROCHA, Ehrenberg.

17. *M. alboflavicans*, Ehrenberg.

With *Lacinularia*, less common, colonies smaller.

CONCHILUS, Ehrenberg.

18. *C. volvox*, Ehrenberg.

Few colonies seen from marsh-water.

FAMILY III. PHILODINADÆ.

PHILODINA, Ehrenberg.

19. *P. roseola*, Ehrenberg.

Common on all sorts of aquatic vegetation.

20. *P. citrina*, Ehrenberg.

Not uncommon, with the last.

21. *P. megalatrocha*, Ehrenberg.

Common.

22. *P. aculeata.*

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Not uncommon among roots of duck-weed in sheltered situations.

ROTIFER, Schrank.

23. *R. vulgaris*, Schrank.

Abundant.

24. *R. tardus*, Ehrenberg.

Among algæ.

25. *R. macroceros*, Gosse.

Common in the forks of *Utricularia* partly surrounded by debris.

26. *R. macrurus*, Schrank.

Many seen.

CALLIDINA, Ehrenberg.

27. *C. elegans*, Ehrenberg.

Several taken among weeds in the marshes. It was so restless that I do not feel sure of the identification.

FAMILY VI. ASPLANCHNADÆ.

ASPLANCHNOPS, de Guerne.

28. *A. myrmelio*, Ehrenberg.

Few were collected in marsh-water. I do not find the ovary so large as figured, but of characteristic horse-shoe shape. I find the trophi to agree well with the descriptions.

FAMILY VII. SYNCHÆTADÆ.

SYNCHÆTA, Ehrenberg.

29. *S. tremula*, Ehrenberg.

Rare in marsh-water. I am not wholly satisfied that it is *tremula*.

FAMILY VIII. TRIARTHRADÆ.

POLYARTHRA, Ehrenberg.

30. *P. platyptera*, Ehrenberg.

Common in water-supply and among aquatic plants.

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TRIARTHRA, Ehrenberg.

31. *T. longiseta*, Ehrenberg.

Not uncommon in the water-supply. The setæ of one were found to be more than three times the length of the body.

FAMILY IX. HYDATINADÆ.

PLŒSOMA, Herrick.

32. *P. lenticulare*, Herrick.

A few dead ones in sediment from water-supply.

FAMILY X. NOTOMMATADÆ.

NOTOMMATA, Gosse.

33. *N. aurita*, Ehrenberg.

Among growing water-plants in clear water. Several seen.

34. *N. lacinulata*, Ehrenberg.

Very common among algæ.

COPEUS, Gosse.

35. *C. cerebrus*, Gosse.

Under *Nelumbium* leaves.

PROALES, Gosse.

36. *P. decipiens*, Ehrenberg.

In water from marsh.

37. *P. gibba*, Ehrenberg.

Not uncommon among roots of duck-weed growing in shallow pools along edges of the marshes.

38. *P. sordida*, Gosse.

Same localities as the preceding species.

FURCULARIA, Ehrenberg.

39. *F. longiseta*, Ehrenberg.

Marsh water.

Numerous species of this family besides those mentioned were found and studied, but the identification was not satisfactory. They may wait for a future opportunity.

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FAMILY XI. RATTULIDÆ.

MASTIGOCERCA, Ehrenberg.

40. *M. carinata*, Ehrenberg.

Among algæ. I have not found the ruby-colored variety common at Corunna, Mich., and which Jennings found in Lake St. Clair.

41. *M. bicornis*, Gosse.

Among duck-weed along shore. Not common.

42. *M. lata*, Jennings.

Rather common in filterings from water-supply.

RATTULUS, Ehrenberg.

43. *R. sulcatus*, Jennings.

Few under floating *Nelumbium* leaves.

CŒLOPUS, Gosse.

44. *C. porcellus*, Gosse.

Common.

45. *C. tenuior*, Gosse.

Common in sediment from marshes.

FAMILY XII. DINOCHARIDÆ.

SCARIDIUM, Ehrenberg.

46. *S. longicaudatum*, Ehrenberg.

Very common.

STEPHANOPS, Ehrenberg.

47. *S. lamellaris*, Ehrenberg.

Common among water drained from duck-weed, and among algæ.

48. *S. chlæna*, Gosse.

Several seen among *Utricularia* from marsh.

FAMILY XIII. SALPINADÆ.

SALPINA, Ehrenberg.

49. *S. brevispina*, Ehrenberg.

Very common among bladderwort and duck-weed.

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50. *S. ventralis*, Ehrenberg.

Common with the preceding.

FAMILY XIV. EUCHLANIDÆ.

EUCHLANIS, Ehrenberg.

51. *E. dilatata*, Ehrenberg.

Common among shore plants.

52. *E. triquetra*, Ehrenberg.

With the preceding.

FAMILY XV. CATHYPUADÆ.

CATHYPNA, Gosse.

53. *C. luna*, Ehrenberg.

Common among algæ in quiet pools.

DISTYLA, Eckstein.

54. *D. Ohioensis*, Herrick.

Not common; among roots of *Spirodela polyrrhiza*.

MONOSTYLA, Ehrenberg.

55. *M. lunaris*, Ehrenberg.

Common.

56. *M. bulla*, Gosse.

Common among vegetation everywhere.

57. *M. quadridentata*, Ehrenberg.

Less common than the last; found with it.

FAMILY XVI. COLURIDÆ.

COLURUS, Ehrenberg.

58. *C. deflexus*, Ehrenberg.

Very common.

METOPEDIA, Ehrenberg.

59. *M. lepadella*, Ehrenberg.

Abundant.

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60. *M. solidus*, Gosse.

Rare.

61. *M. oxysternum*, Gosse.

Few seen.

FAMILY XVII. PTERODINADÆ.

PTERODINA, Ehrenberg.

62. *P. patina*, Ehrenberg.

Common.

63. *P. reflexa*, Gosse.

Rare.

FAMILY XVIII. BRACHIONIDÆ.

BRACHIONUS, Ehrenberg.

64. *B. militaris*, Ehrenberg..

Very common, in water-supply and among plants along shore.

NOTEUS, Ehrenberg.

65. *N. quadricornis*, Ehrenberg.

Few in shallow pools mantled by Lemna.

FAMILY XIX. ANURÆADÆ.

ANURÆA, Gosse.

66. *A. cochlearis*, Gosse.

Extremely abundant in water-supply of Sandusky.

67. *A. stipitata*, Ehrenberg.

Abundant in the water supply of Sandusky.

Nearly or quite as abundant as the last; readily separated by its shape as shown by Gosse and by the tessellation of the dorsal shield. In *A. cochlearis* there is a dorsal keel extending from posterior spine to the cervical plate; in the other, the two middle rows of dorsal plates are alternately and irregularly hexagonal and pentagonal, breaking the dorsal ridge.

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THE REQUISITES OF A PURE WATER-SUPPLY.

WILLIAM C. KRAUSS, M. D., F. R. M. S., BUFFALO, N. Y.

In the rapid growth characteristic of our American cities and villages, the question of adequate water supply becomes one of the great and pressing needs for early consideration, and in many communities natural or artificial sources are selected more with the view of supplying *quantity* rather than *quality* of water. Even should these sources have been of unquestioned purity the improper disposal of the city's sewage, through the stupidity or carelessness of the proper officials, may have exposed the water to contamination and rendered it in time dangerous and polluted.

Another phase of this question is seen in cities drawing their supply from natural waterways upon which they may be located, in the slowly and gradually decreasing standard of purity, due to the refuse-disposal of rapidly growing cities or villages situated farther toward the source of these same water-courses.

This important and vital question of pure water supply and means of retaining it, is just now attracting timely attention by scientists and engineers, especially in the Middle and Eastern States of our Country. The ravages and immense cost of epidemics through water-borne or water-bred specific germs have awakened many communities to a proper sense of the impending and threatening danger awaiting them, and are devising ways and means to avert the inevitable results.

Just what a pure supply means is hardly open to much debate and may be summed up in five propositions, as follows:

1. That the water-supply of any city or village should

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not in any possible way be liable to pollution or contamination from the sewage of any other community.

2. That the sewage of a city should not be emptied into any water course not having a current of three to five miles per hour, and then the sewage entrance should be at a distance one mile or more from the intake.

3. When the water-supply of any city or village is a navigable stream the water should be sand-filtered before pumped into the city reservoirs or water mains.

4. That for ordinary drinking purposes the water should not be taken in its primitive or raw state, but be either filtered, boiled or distilled and aerated.

5. That not only chemical but bacteriological examinations of the water should be made, at least once weekly, to determine its character as a safe or dangerous water for domestic use, and where contamination is shown to exist, the services of an engineer be enlisted to detect, if possible, the cause and origin of such contamination.

This last proposition is the one to which I wish to call the attention of the society today. Who is to decide whether a water is potable, the chemist or the bacteriologist? Up to within a few years ago a chemical examination of water was deemed sufficient to decide its potability, and upon the decree of the chemist the water was either accepted or rejected.

The chemist was generally able to detect the presence of decomposing organic matter, either vegetable, animal, or both, which in his analysis was indicated by the presence of ammonium compounds and the oxygen consuming power of the water. The ammonium compounds, particularly the albuminoid ammonia are usually the result of putrefactive fermentation of nitrogenous matter, and water of high purity should contain from none to .041 parts per million, while in impure water it ranges from .082 and over.

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The consumption of oxygen by the water and the formation of carbon dioxide occurs during the fermentation of small quantities of organic matters in the water, and provides a most delicate indication of the presence of such matters in a suspected water. The following proportions are given by Frankland and Tidy, as the basis of interpreting the results of this method:

High organic purity	0.05 parts per million (in three hours).
Medium purity	0.5 to 1.5
Doubtful	1.5 to 2.1
Impure	2.1

The presence of chlorine and phosphoric acid must also be regarded with suspicion when found in large amounts, as they are constant ingredients of animal excretions. Surface waters ordinarily contain but a few parts per million.

The chemist, then, is only able to say that a water contains organic matter in the process of fermentation, but cannot say how virulent or innocent are these destructive agents. It is now generally accepted that organic matters, which by one means or another find their way into surface waters are oxidised and eventually reduced to simple substances by the operations of microörganisms, and not by mere chemical changes independent of them. In other words, the oxidation of impure, polluted water is the result of bacterial activity, and the decree of the bacteriologist is now imperative in deciding the pathogenic or non-pathogenic character of the bacteria.

The number and variety of species present in water from any given source will depend upon conditions relating to the amount of organic pabulum, the temperature, the depth of the water, the fact of its being in motion or at rest, and its pollution from various sources.

The water from artesian wells contains no bacteria, while that of sluggish streams, lakes and rivers receiving the sewage of large cities contains millions of colonies per cubic centimeter. Authorities consider a water having 250 bacteria per cubic centimeter, or less, as entirely safe and usable.

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It is now generally recognised that the mere enumeration of the number of colonies which develop from a water under investigation is not a sufficient indication upon which to found an opinion as to its potability. Of course, the greater the number of colonies the more organic pabulum is present for these microörganisms. The bacteriologist is not able as yet to give any definite idea of the amount of such organic matter, while the chemist is able to do so with considerable precision. But the bacteriological examination may prove to be of great value if it succeeds in demonstrating the presence of certain pathogenic bacteria, and in thus preventing the use of a dangerous water. Moreover, the number of colonies is an index of the probable quantity of organic matter which may come from a dangerous source; and the dangerous pathogenic bacteria are not only likely to be present in such water but they can more readily multiply in it. (Sternberg.) Sternberg gives the number of varieties of non-pathogenic micrococci found in water as thirty, pathogenic micrococci, two—the *Staphylococcus pyogenes aureus* and the *Micrococcus Biskra.* Of the non-pathogenic bacilli seventy-nine varieties have been found, while of the pathogenic bacilli sixteen varieties, including the bacillus of typhoid fever, of cholera, and the *Bacillus coli communis.* These three varieties are the most important findings possible of a bacteriological examination and are positive proof of the presence of alvine dejections in the water.

The continued presence of typhoid fever in a municipality is as inexcusable as vermin in a modern dwelling, and indicates a degree of shiftlessness and apathy not consistent with modern American views and energy.

Lake Erie, the water supply of Buffalo, also of the cities of Toledo, Cleveland, Dunkirk, Erie, Ashtabula, is subject to perfodical upheavals, due to its shallowness and the strong west and northwest winds occurring during the winter and spring months. As a result we have roily water five or six months of the year, beginning with November or December and lasting until May or June. It will be interesting,

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therefore, to compare the results obtained by the city chemist and city bacteriologist, both very capable and scientific gentlemen, and see how they agree or disagree, with a view of determining which is the more trustworthy guide. These examinations are made daily by the bacteriologist and monthly by the chemist, under the direction of the health commissioner:

DECEMBER, 1895.

*Chemical Analysis:**

"Organic residue, 3.36 grains per gallon; albuminoid ammonia, 0.078 parts per million; oxygen dissolved in four hours, 0.62 parts per million. Conclusion—Water continues in excellent condition."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 765; lowest number of bacteria per cubic centimeter, 55; average number of bacteria per cubic centimeter, 225; in reservoir, 10. The bacillus Janthinus was found on December 18th and 23d, indicating probable filth contamination. Conclusions—Water is in poor condition."

During December large quantities of dead fish were found along the south shore of Lake Erie.

Enteric fever deaths, 11. Death-rate, 11.65 per 1,000.

JANUARY, 1896.

Chemical Analysis, January 28, 1896:

"Organic residue, 2.72 grains per gallon; albuminoid ammonia, 0.148 parts per million; oxygen absorbed in four hours, 0.43 parts per million. Conclusions—This water is in good condition."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 3,250; lowest number of bacteria per cubic centimeter, 195; average

* These are taken from the monthly reports of the Board of Health and are the official figures.

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number of bacteria per cubic centimeter, 800. Bacillus Janthinus present on January 3d. At that time and during the week following the water was in very poor condition. A gradual improvement is noted."

Death-rate, 11.68 per 1,000. Enteric fever deaths, 5.

FEBRUARY, 1896.

Chemical Analysis, February 28, 1896:

"Organic residue, 1.96 grains per gallon; albuminoid ammonia, .092 parts per million; oxygen dissolved in four hours, 0.56 parts per million. Conclusions—Water is good."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 8,820; lowest number of bacteria per cubic centimeter, 60; average number of bacteria per cubic centimeter, 1,000. Tap water figures excessive, due to storms, dirty ice and low water in reservoir."

Death-rate, 10.61 per 1,000. Enteric fever deaths, 3.

MARCH, 1896.

Chemical Analysis, March 28, 1896:

"Organic residue, 3.31 grains per gallon; albuminoid ammonia, 0.077 parts per million; oxygen absorbed in four hours, 0.510 parts per million. Conclusions—This water is in excellent condition."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 990; lowest number of bacteria per cubic centimeter, 65; average number of bacteria per cubic centimeter, 260. In the reservoir there were found, on March 2d, 2,205, and on March 13th, 19,790 bacteria per cubic centimeter, due to slush. Conclusions—The condition of the water is good."

Death-rate, 11.58 per 1,000. Enteric fever deaths, 4.

APRIL, 1896.

Chemical Analysis, April 28, 1896:

"Organic residue, 2.67 grains per gallon; albuminoid

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ammonia, 0.032 parts per million; oxygen absorbed in four hours, 0.302 parts per million. Conclusions—This water is in excellent condition."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 20,160; lowest number of bacteria per cubic centimeter, 80; average number of bacteria per cubic centimeter, 3,000. Conclusions—Large bacterial contents during this month, due to quantities of dirty ice. The water is not in good condition, but no harmful or polluted water organisms have been found."

Death-rate, 12.67 per 1,000. Enteric fever deaths, 4.

MAY, 1896.

Chemical Analysis, May 28, 1896:

"Organic residue, 3.24 grains per gallon; albuminoid ammonia, 0.088 parts per million; oxygen absorbed in four hours, 0.55 parts per million. Conclusions—This water is in poorer condition than last month. There is evidence of contamination."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 18,900; lowest number of bacteria per cubic centimeter, 230; average number of bacteria per cubic centimeter, 2,300. Conclusions—Ice stopped running on May 7th, after which time the bacterial contents gradually lowered until the 14th. On this date and the day following the number increased, due to wind. At present time (May 26th) water is in good condition (270 per cubic centimeter)."

Death-rate, 11.40. Enteric fever deaths, 1.

JUNE, 1896.

Chemical Analysis, June 29, 1896:

"Organic residue, 3.97 grains per gallon; albuminoid ammonia, 0.087 parts per million; oxygen absorbed in four hours, 0.659 parts per million. Conclusions—This water

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has improved somewhat in quality since the last examination."

Bacteriological Examination:

"Highest number of bacteria per cubic centimeter, 700; lowest number of bacteria per cubic centimeter, 35; average number of bacteria per cubic centimeter, 235. Conclusions —The water is in good condition."

Death-rate, 11.79 per 1,000. Enteric fever deaths, 3.

During December, although the organic matter in the water was small and the number of bacteria correspondingly so, yet there was found a bacillus which is generally found in water which has undergone sewage contamination. During this month there were eleven deaths reported from typhoid fever. The bacillus of typhoid was not found in the water, although repeated examinations were made with this end in view. It is, however, extremely difficult to detect the typhoid bacillus, as the following extract, quoted from Rafter* will prove:

"Messrs. Laws and Andrewes† state that working on ordinary London sewage they failed in every case to recognise the typhoid bacillus, until finally the failures were so numerous that they became oppressed by a sense of mathematical improbability. Thus the average amount of sewage produced in London amounts to 200,000,000 gallons per day. During June, 1894, while the investigation was in progress, 177 cases of typhoid fever were notified in London, to which may be added thirteen cases of continued fever, making 190 cases in all. Adding something for cases not reported and Messrs. Laws and Andrewes conclude that during June, when typhoid is by no means prevalent, it may be assumed that there were 200 cases in all. Some of these suffer from constipation and hence contribute very little fecal matter to the general mass of sewage. For these and other reasons cited, any estimate

* George W. Rafter, on Lake Erie as a Water Supply, *Buffalo Medical Journal*, August, 1896.

† Report on the Result of Investigation on the Microörganisms of Sewage. By J. Parry Laws and F. W. Andrewes, 1895.

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of the average amount of sewage contributed daily by typhoid cases in London must be purely conjectural, but at a reasonable estimate it is placed at two hundred and fifty millionths of the whole, and, as Laws and Andrewes point out, every endeavor is made to disinfect this before it is allowed to pass into the sewers. The mathematical chances of detecting the typhoid bacillus in ordinary London sewage are, therefore, extremely remote. Assuming the typhoid bacillus to be intimately mixed with the ordinary sewage there would be only one typhoid bacillus in one-tenth of a cubic centimeter of sewage at the outfalls.

"But the investigators only found it possible to work on one five-thousandths of a cubic centimeter, and this only when 90 per cent. of the organisms were inhibited by the addition of 0.05 per cent. carbolic acid and incubation at 37° Cent.

"These considerations were so discouraging that it was determined to work on sewage from a fever hospital, and arrangements were accordingly made to allow the dejections from forty patients in the Eastern Hospital, at Homerton, to pass into the hospital sewer for two days without disinfection. A series of samples were then taken and cultivations made therefrom at once. Without going further into detail, it may be stated that from the whole series of sewage taken under these circumstances only two colonies of the bacillus of typhoid fever were certainly differentiated. In their summation Messrs. Laws and Andrewes say:

"'We must be content to have shown that in the drain from the typhoid block of a fever hospital, when the stools had not been disinfected for two days, a bacillus can be found which, so far as demonstration can go, is identical with that believed to be the actual cause of typhoid fever. So far as we are aware, this important fact has never been previously demonstrated.'"

In January, according to the city chemist, the water was in good condition, while the bacteriologist again found the bacillus Janthinus and reported the water to be in "very

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poor condition" for the first half of the month and a gradual improvement the last half. The number of typhoid deaths decreased to five. In February and March both officials found the water in good condition, also evidenced by the death-rate and the typhoid deaths, three and four respectively.

For the months of April and May there exists a disparity between the two reports, the chemist claiming that the water was in "excellent condition" during May, while the bacteriologist found the water "not in good condition," because of large bacterial contents. The deaths from typhoid fever were four—an unusal time for typhoid to be present. For May the chemist found evidences of contamination, while the bacteriologist, on the preceding day, considered the water in good condition, containing on that day only 270 bacteria per cubic centimeter. In June both officers found the water in good condition.

Reviewing the opinions of the city chemist and the city bacteriologist, it is evident that the disparity occurs whenever the water is in an improper and unhealthy condition, and, when such is the case, it is only fair to assume that some error has been committed favoring the water.

The bacteriological examination as well as the chemical analysis are, therefore, alike necessary in demonstrating the purity and safety of a drinking water, and neither should be omitted when the least suspicion exists as to its contamination. The consumers should be immediately warned of the dangers of using such water in its raw state, and should be enjoined upon to make some attempt at purification, either filtering, boiling or condensing. This applies not only to those cities whose water-supply is open to contamination but to every city having a public supply.

Besides the chemist and bacteriologist, the water department of every city, whose supply is liable to contamination, should have the services of an engineer, whose fame does not rest upon his theoretical knowledge and bureaucratic propensities, but upon his practical information of the laws of hydrology and hydrodynamics. Such an one, well versed in the

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hydrography of his locality, could almost prognose the condition of his water-supply and be an important aid to the city's health department. As such he would be able to render the same valuable services, as does the Local Forecaster in meteorology, and give warning several days beforehand of marked changes in the quality of the water.

While the chemist and bacteriologist are only able to detect impurities after contamination, the engineer could foresee these changes and give sufficient warning, or attempt to overcome the impending contamination before the city mains and reservoir are filled with the poisonous liquid.

The question of food contamination and adulteration has attracted the attention of the lowest tribunal up to the highest (Filled-cheese bill of last Congress), and severe laws are upon the statute book punishing offenders, and yet the water-supplies of some of our cities are openly and flagrantly polluted and no attempt whatever is made at punishment, partly because insufficient attention has been given to this most important subject. The time, however, may not be far distant when the same rigid inspection and the same jealous care will be extended to one of the most important adjuncts to the life, health and comfort of every individual, of every society and of every commonwealth.

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PUBLIC WATER SUPPLY FOR SMALL-TOWNS.

M. A. VEEDER, M. D., LYONS, N. Y.

Drinking water that is manifestly bad does not make everyone that uses it sick. Even when the mains and reservoirs of a public water-system have been infected by such a poison as that of typhoid it is only exceptionally and for limited periods that as many as one per cent. of those using the water contract the disease. An outbreak of 2,000 cases in a population of 200,000 is ordinarily regarded as a severe epidemic, and yet this is at the rate of only one person in a hundred. It is this immunity on the part of the great mass of the people that permits infected systems of water-supply to continue in operation. If there were no resisting power on the part of the individual, all would die on the slightest exposure and the source of danger would soon be thoroughly identified and avoided. As it is, however, for every one that contracts the disease there may be as many as a hundred who escape. Thus it becomes a question of probabilities, and there is a chance for much plausible theorising and controversy. Gradually, however, as the result of increasing observation and experience, crude ideas that have prevailed are being eliminated and the truth of the matter established.

Only a few years ago the most essential point in the improvement of water-supply was thought to be the determination of the chemical ingredients held in suspension or solution. Elaborate systems of analysis were devised for this purpose, and the quality of the water was judged almost entirely by its chemical reactions. Thus it became customary to consider the questions involved from a chemical point of view exclusively. The simple dilution of contained matters of a chemical nature, if carried far enough, would make them

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harmless. Consequently large bodies of water were supposed to have a power of self-purification in direct proportion to their size. In like manner precipitation, sedimentation, aeration and other chemical and mechanical processes were supposed to have a purifying effect. The quantity of sewage entering a stream being known, it becomes possible to tell with a good degree of certainty at what distance, mingled with such a volume of water, it will become so diluted, diffused and changed as to be unrecognisable by any chemical test. The dose of poisonous matter, if of a chemical nature, ought to be divided and subdivided to such an extent as to be entirely harmless in the quantity of water that any individual would consume. In practice, however, this is not found to be the fact, sewage infection being capable of producing epidemic disease for many miles along a stream entirely out of proportion to any possible chemical process of diffusion.

The whole tendency of modern research has been to show that the question as to the spread of disease through the agency of water is biological rather than chemical. It is the presence of certain living organisms and of the conditions on which their continued existence depends that leads to the spread of disease. A single seed may be the means of overspreading an entire continent with some form of luxuriant growth, and so a single disease germ may start an epidemic, not through any mechanical or chemical process of division or subdivision, but because having life it grows and multiplies.

The danger consists not in the quantity of such organisms but in their power of growth under given conditions. If capable of living in water, they may infect an entire stream instead of disappearing by processes of dilution within a few rods. Unlike chemical poisons, they have no fixed poisonous dose. The smallest possible inoculation may prove fatal through the power of self-propagation which they possess. If, on the other hand, their growth be hindered by unfavorable temperature, moisture, or food supply, they may become harmless no matter what their quantity. It is true that they

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have chemical effects, originating substances known as toxines, some of which are deadly poisons, but they themselves depend upon the possession of life for the modes of activity which they exhibit. Throughout it is a question of vitality under particular surroundings.

Typhoid fever, cholera and certain forms of dysentery are the chief diseases whose infection it is generally admitted can live in water. In addition, about ten years ago, the writer came to the conclusion that the term malaria, signifying bad air, is a misnomer, and that diseases of this class are very largely, if not exclusively, conveyed in water. Towns taking their public water-supply from ponds or streams having distinctly malarial surroundings have become subject to such fevers although previously free from them.

The manner of spreading of the diseases which have been named originates two classes of dangers. If water be taken from the vicinity of human habitations there is liability to contamination from excreta washed into the pond or stream used as a source of supply, or, in the case of wells, the strong action of powerful pumps may originate a rapid flow underground extending many hundreds of feet and carrying impurities through coarse gravel or open crevices in the soil. That this is the fact appears from the manner in which ordinary wells at a considerable distance from the pumping station run dry when the latter is in operation. Such contamination from human sources may originate typhoid and diarrheal disorders. If, on the other hand, the source of supply is remote from human habitation there may be malarial contamination. Indeed the natural habitat of malaria is in new and undrained countries and virgin soil. In view of this distribution of the disease it is surprising that well-drained cities, having perfect sewers, should yield a certain percentage of malarial fevers until the source of their water-supply is noted, it being in such cases, as a rule, some pond or stream in whose vicinity these diseases are prevalent. Shallow wells in alluvial soil also may yield malarial infection. It is said that since the substitution of deeper artesian borings

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for such wells there has been a notable decrease of malarial diseases in some parts of the Southern States of North America.

In many localities it is difficult, if not impossible, to secure an adequate supply of water free from the forms of contamination to which reference has been made. This necessitates some system of purification.

It has been discovered recently that there is an antagonism between disease germs and what are known as nitrifying organisms, which produce nitrites and nitrates in the soil. Advantage has been taken of this to institute an intermittent process of filtration. Water containing the bacteria that it is desired to destroy is allowed to run into a filter composed of sand, containing an abundance of nitrifying organisms, and instead of being drawn off immediately is allowed to stand for a sufficient length of time to permit the destruction of the disease germs by their natural foes.

Such filtration as that just described is but the perfecting of natural processes. Alternation of rainfall and dry weather operates substantially on the same plan, tending to purify the ground water in the soil from infection and making wells possible. Thus in localities where artificial filter beds are impracticable it may be possible to resort to wells with similar results. Experimental borings are required in order to determine whether the quantity of water is adequate and whether the soil through which it percolates is adapted to secure its purification. This being done and the system established, the intermittent action of the pumps, running a part of each day, like intermittent filtration, yields a much purer supply than could be had in any other way. A point to be guarded against is the influx of surface water, which is specially liable to contain malarial infection as well as other impurities. To this end, numerous small wells, consisting of iron pipes put down to the proper depth and having perforations over a space of six or eight feet from their lower extremities, covered with fine wire gauze, may be employed. Another plan that may serve is to have a single large well, twenty feet or more

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in diameter. A convenient method of construction of such a well is by the use of a curb, built up in a hexagonal or octagonal form, of plank laid flatwise and spiked one upon the other in layers. If such a curb be made, slightly smaller toward the top, it can be carried down successfully through almost any sort of soil and stoned up.

It has been thought best to enter somewhat into such details as have been indicated, because they illustrate the principles involved in improvement of water supply, especial reference having been had throughout to localities whose resources are limited. The adaptation of laboratory results to practical uses is the point specially sought to be accomplished in this brief summary. The sanitary engineer, the practising physician and the skilled microscopist are upon common ground in these studies.

At the present stage of progress it must be admitted, however, that serious imperfections are unavoidable in the best systems of water-supply available in many localities. This being the case, household methods of purification require to be taken into the account. That preferred by the writer is as follows: The water is boiled and allowed to stand in a covered stone jar until all sediment has deposited. It is then transferred to ordinary air-tight glass fruit jars, a lot of which, having convenient modes of fastening, are kept for the purpose. When put in an ice chest or cool cellar such water comes out beautifully clear, sparkling and palatable. Such water has no unpleasant flavor unless kept too long, and even this might be avoided by sterilising the jars and filling them with the water while hot, which would require reheating after the sediment is removed. Practically there is no necessity for this extra trouble. Certainly all the waters treated by the writer in this way have proved to be excellent, and there can be no question as to their freedom from the infection of any of the diseases that have been named in the discussion. It may be noted also that substantially the same principle is employed when water is used for quenching thirst in the form of tea, coffee, soups and the like. It is the boil-

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ing that makes such waters safe, the various ingredients added serving to please an acquired taste for the most part. Mankind is accustomed to take many precautions of this sort without any clear ideas of the reasons. It is the province of advancing civilisation to enable such precautions to be taken intelligently, and consequently more perfectly, and this is the aim of the present discussion in regard to water-supply.

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THE INCREASING POLLUTION OF OUR MUNICIPAL WATER-SUPPLIES.

FRANK J. THORNBURY, M. D., BUFFALO, N. Y.

It seems hardly necessary here to go into a detailed account of the necessity for a pure drinking water. The same is also precluded by the length of the paper which I have prepared. Certainly no agent is more important to the well-being of the human race—a fluid which constitutes about three-fourths of the human body and animal tissues generally; the universal solvent; the beverage of beverages, essential to the performance of all the vital functions. As vegetation on the earth's surface would be impossible without moisture, so also would the human body soon cease in its vital phenomena without an adequate supply of hydrogen and oxygen associated in their proper proportions. While it is essential for the performance of these offices it is equally necessary in removing the results of functional activity—namely, waste tissue products. This brings us to the consideration of *purity*. As water can hold in solution only a certain amount of solids, its solvent power in the system—one of its chief effects—is necessarily impaired when it is charged with compounds, organic and inorganic, and the waste products which it should take up and carry off through the emunctories, accumulate and exert their deleterious effects, both mechanical and chemical.

While the system may combat for a time a certain excess of mineral impurities, they sooner or later make their effects manifest upon the excretory organs, the kidneys especially. Just how much of a rôle the lime salts carried by water have

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in the causation of the pipe-stem arteries of advancing life, in calcification and atheroma, is not fully established, but the inference seems justifiable that such influence is considerable. These changes conform to the pathological histology of contracted and granular kidney the etiology of which is as yet obscure, to the general condition of the circulatory system known as arterio-capillary fibrosis. It being through the circulatory system that water is carried through the body, and constituting, as it does, about nine-tenths of the weight of the blood and fluids therein contained, naturally it is in the arterial and capillary systems that the effects of impurities thus carried are apt to be first noticed. Probably the chief primary effect is simple irritation, as this would explain best the microscopical changes characterising arterio-capillary fibrosis.

So much with reference to the general circulatory effect of the *inorganic* impurities upon the system. The chief feature of impure water which I wish to discuss, pertains to its *organic* constituents, not chemical alone but biological; to the living pathogenic microörganisms which it is liable to contain. I do not wish to enter into the effects of albuminoid ammonia and other chemical products indicating serious pollution of a water-supply, but rather prefer at this time to confine myself to the subject of the bacterial contamination, introducing, however, a few general considerations.

Undoubtedly the contamination of our municipal water-supplies is at the present time reaching a degree which should occasion great alarm, and no one who has given the subject thought can fail to be impressed with the importance of a consideration of the ways and means of suppressing the evil. It is equally true that more ailments and diseases arise from the use of impure water than from any other known cause, and yet most people are conspicuously careless as to the quality of water which they drink. In many cases this is the result of ignorance; in others it is due to carelessness.

Thus the subject becomes the most important sanitary topic which we have to consider today, and thus far it has not

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received the amount of attention which it merits. The condition referred to is the natural outcome of the rapidly increasing population of our country, without corresponding increase of precaution in the disposition of sewage, which in most instances is allowed to mingle with adjacent waters unaltered. "Even a small amount of sewage entering near the intake to a water-supply is sufficient to destroy the small margin of safety otherwise existing in regard to the water's purity," (Rafter) and hence, as may readily be understood, there are as yet few sanitarily unobjectionable water-supplies in the United States. In many cities the condition of affairs is really alarming, as attested by frequent outbreaks of typhoid fever, or, as is the case in other instances, by the endemic presence of this disease. Among such cities may be mentioned New York, Chicago, Philadelphia, Cincinnati, Pittsburg, St. Louis, Buffalo, Cleveland, New Orleans, Minneapolis, Milwaukee and Detroit, besides many smaller towns. In all of these and other parts we have frequent controversies concerning the impurity of the local water-supply, and discussions and agitations as to the most available means of improving the condition. In some of the cities active steps have been taken, or are being taken, to abolish or mitigate impending dangers. In others, too much politics and want of proper sense of duty on the part of the authorities lead to a continuance of the deplorable state of affairs.

To give an impression of the kind of water furnished to the inhabitants in some of our large cities the following quotation may be of service:

"A sad state of affairs exists in Brooklyn. The source of the water-supply is inadequate and the daily papers are full of complaint. A lay contemporary, who has taken pains to investigate the matter, speaks as follows: 'From stagnant ponds, vile and reeking, filled with dirt and decaying and fermenting vegetation, covered with slimy decomposing masses, swarming with bugs, insects and fish, full of white snaky threads, receiving gas-house refuse and house drainage, and covered with green scum, we get the water we

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drink.' No wonder the citizens howl, for this is a shameful description of the water resources of a city that is soon to wed New York."

Naturally, sewage constitutes the most important source of pollution of our municipal water-supplies. Could some means of its treatment on a large scale (other than the sewage farm system), as, for example, electrolysis, be devised, much would be done toward solution of that much vexed question, the purification of our municipal waters. As in preventative medicine, prophylaxis is the first law of sanitary science today.

Chicago discharges about 50,000 cubic feet of sewage per minute into Lake Michigan, through its river, the Chicago. London, England, has 200,000,000 gallons of sewage per day. "Erie, Pa., has 5,000,000 gallons daily, and Toledo, O., 8,000,000. Cleveland, O., in 1893, besides her sewage, emptied 15,693 cubic yards of night soil into Lake Erie and 7,800 tons of garbage. Dunkirk has thirteen miles of sewerage." (Krauss.)

From these figures an impression may be formed as to the amount of sewage and refuse which any town has to dispose of, and we may more fully realise the enormity of this influence in contaminating a body of water naturally pure. This, to reiterate, is one of the worst penalties of increasing population and the aggregation of the masses.

A timely contribution on the subject of sewage was that of Mr. E. O. Jordan, of the Massachusetts State Board of Health, (1889–'90). Mr. Jordan reports on and carefully describes twelve species of bacilli, most of them previously unknown, isolated at the experimental sewage station of Lawrence, Mass., and further adds an important report on nitrification and the organisms concerned in the process.

The results of Mr. Jordan's examination of the sewage of Lawrence, Mass., gave an average of 708,000 living bacteria per cubic centimeter, his highest result being 3,963,000 per cubic centimeter. He obtained far greater numbers during the summer months than at other times. Laws and Andrewes, from whose report I take the liberty to quote, giving them due

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credit, found that the number of bacteria in London sewage is considerably higher than the above. Determinations of the number of microörganisms in the sewage of the King's Scholar's Pond sewer, London, in November and December, 1891, showed 2,618,000 and 3,179,000 respectively. In their report to the London County Council the last-named observers state that "in perfectly fresh sewage from St. Bartholomew's hospital on January 26, 1894, at 10.30 A. M., from which cultivations were made immediately, without allowing any time for the multiplication of the microörganisms, we found on an average 2,781,650 bacteria per cubic centimeter. Two agar-agar plates, inoculated each with 1 cubic centimeter of the sewage diluted 10,000 times and incubated at 22° C., yielded respectively 216 and 330 colonies." Six similar plates, inoculated each with 1 cubic centimeter of the sewage diluted 100,000 times, yielded respectively 21, 39, 26, 27, 28 and 29 colonies. The same sewage, however, after being kept for three days in a stoppered bottle, showed an enormous increase in bacteria; a similar plate, inoculated with 1 cubic centimeter of the sewage diluted 100,000 times, yielded 388 colonies, representing 33,800,000 bacteria per cubic centimeter.

From the Fleet sewer on Snow-hill, London, the results were somewhat higher. The sewage was taken on March 2, 1894, at 11.30 A. M., and cultivations were made immediately. Three agar-agar plates gave 40, 28 and 34 colonies, yielding an average of 3,400,000 bacteria per cubic centimeter. With this sewage also further experiments were made to ascertain what proportion of the bacteria present would grow at a temperature of 37° C., or with the addition of .05 per cent. carbolic acid, or under the influence of both these conditions combined. Two agar-agar plates, inoculated each with a cubic centimeter of the sewage diluted 10,000 times, with the addition of .05 per cent. carbolic acid, and incubated at 37° C., yielded respectively 59 and 38 colonies, giving an average of 485,000 organisms which could grow under these unfavorable conditions.

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"These results indicate that a temperature of 37° C. inhibited the growth of 73.4 per cent. of the total number of organisms present in this sewage, while the addition of .05 per cent. carbolic acid inhibited the growth of 44.2 per cent. of the total number; the combination of .05 per cent. carbolic acid, with a temperature of 37° C., inhibited the growth of no less than 85.8 per cent. of the bacteria present.

"Two agar-agar plates, containing each 1 cubic centimeter of the sewage diluted 100,000 times, but with the addition of .05 per cent. carbolic acid, yielded, when incubated at 22°, 11 and 8 colonies respectively, equal to 950,000 organisms per cubic centimeter of sewage able to grow in the presence of this amount of carbolic acid, *i. e.*, 50 per cent. were inhibited in their growth.

"With regard to the total number of organisms present in sewage, the highest results were obtained from a sample taken at the Crossness outfall, London, England, at 2.30 P. M., of July 10, 1894. Six agar-agar plates were made, each containing 1 cubic centimeter of the sewage diluted 100,000 times, and they were incubated at 22° C. They yielded respectively 124, 127, 105, 119, 110 and 88 colonies, or an average of 11,216,666 microörganisms per cubic centimeter of sewage." (Laws and Andrewes.)

Can one conceive how even great dilution of such sewage in water can render it free from danger?

"The sewage collected from the fever hospital at Homerton, London, on May 23d, at 11 A. M., was examined chiefly for the purpose of discovering the typhoid fever bacillus.

"No less than forty-five colonies resembled, at first sight, *B. coli communis*, but of these less than a dozen responded to the chemical tests (coagulation of milk, formation of gas bubbles in gelatine shake-cultures, and production of indol in broth,) which are relied on to distinguish the bacillus from its allies. It was, nevertheless, if we except a certain streptococcus found, the commonest, and certainly the most conspicuous, of the organisms present in this sewage. Those of the forty-five colonies which failed to give some or all of the

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three chemical tests must be classed, from their morphological and cultural resemblances, as close allies of *B. coli communis*, a near relative of typhoid, but we are unable to refer them to any described species.

Proteus Zenkeri, a common putrefactive organism, occurred twice. A bacillus allied to *B. pyocyaneus*, of green pus, which we describe as *Bacillus cloacæ fluorescens*, occurred twice.

The following other organisms were found to be present in sewage in numbers varying from 200,000 to 2,500,000 per cubic centimeter: *Bacillus fluorescens stercoralis*, *Bacillus albus putidus*, *Bacillus fluorescens liquefaciens*, *Bacillus cloacæ fluorescens*, *Bacillus mycoides*, *Proteus cloacinus*, *Proteus Zenkeri*, a streptococcus coagulating milk, *Staphylococcus pyogenes citreus*, *Sarcina flava* and its allies, and *Diplococcus albicans tardissimus*. Other bacilli which rapidly liquefy gelatine and produce a green fluorescence, were found in numbers varying from 10,000 to 200,000 per cubic centimeter."

The method employed by Laws and Andrewes in their study of sewage, which was designed to secure an equable admixture of the material with the large amount of diluent required, was as follows: Ten cubic centimeters of the mixed and shaken sewage were diluted to 100 cubic centimeters with recently boiled distilled water in a sterilised and accurately stoppered flask, and thoroughly shaken. Of this dilution 10 cubic centimeters were taken and further diluted to 1,000 cubic centimeters with sterilised distilled water in a second sterile flask, and again well shaken. Of this second dilution (which represents 1 in 1,000) two further dilutions were made in similar manner—one by taking 10 cubic centimeters and diluting to 100 cubic centimeters (giving a dilution of 1 in 10,000), and another by taking 10 cubic centimeters and diluting to 1,000 cubic centimeters, giving a dilution to 1 in 100,000. These extreme degrees of dilution were, of course, rendered necessary by the enormous number of microörganisms present even in fresh sewage. And even with the

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extreme dilutions, the gelatine plates used for cultivating the organisms soon became useless, on account of the rapid liquefaction produced by the growth of bacterial colonies.

The disinfection of typhoid excreta is a matter of extreme difficulty, and is, as a rule, very imperfectly carried out even in fever hospitals. It hence results that sewage becomes a very potent agent in the dissemination of this disease and the contamination of water supplied by such sewage the chief cause of typhoid epidemics. Attention to the inquiry which the London investigators carried out was especially directed to the possible occurrence of the *Bacillus typhosus* in London sewage, and every colony which seemed likely to belong to this species was the subject of careful investigation.

It is estimated that even though the typhoid fever bacillus be intimately mixed with the city's sewage from typhoid fever cases direct, there will be only one typhoid fever bacillus in one-tenth of a cubic centimeter of the sewage at the outfall. So numerous were the failures of the above observers in their attempt to find the typhoid bacillus in London sewage they finally became oppressed by a sense of mathematical improbability. The average amount of sewage produced in London, England, is 200,000,000 gallons per day. Calculating that 200 cases of typhoid fever prevailed during the time when the observations were made, it is estimated that the amount of typhoid sewage amounted to one two-hundred-and-fifty-thousandths of the whole. The investigators only found it possible to work on one five-thousandth of a cubic centimeter of sewage

The chief interest which attaches to the contamination of drinking water by sewage pertains to the possible presence of the typhoid fever bacillus (or virulent colon bacilli, which produce a train of clinical symptoms closely corresponding with those caused by the former organism). Laws and Andrewes have shown that in the drains from a hospital where typhoid fever is prevalent the typhoid fever bacillus may be isolated without difficulty. This had never previously been done. The organism was, furthermore,

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found to live in raw sewage, at the ordinary temperature, for two weeks. At a warmer temperature, even after thirty days and in presence of pure cultures of various other organisms, the typhoid bacillus was found alive and active. The colon bacillus was cultivated in sewage through several generations.

From the foregoing deductions it would appear that the fact of the typhoid fever bacillus not being found more often in water supplies is no particular argument against its presence. The better method for demonstrating the presence of the typhoid bacillus in water is by the inoculation of white rats with samples of water placed in beef tea and incubated for twenty-four hours at 40° C. The presence also of a small amount of carbolic acid with the higher temperature will restrict the development of most of the water bacteria, when we will have remaining simply typhoid (or colon bacilli). Such cultures injected into the abdominal cavity of a white rat promptly lead to the development of severe inflammatory symptoms or even death. Without this animal experiment the task of finding the typhoid bacillus in drinking water is often exceedingly difficult; with it it becomes comparatively easy. The new serum test, with which I am myself now experimenting, promises to be an important aid.

[This consists in adding to a hanging drop of a mobile organism a drop of blood (even after it has been dried) from a patient suffering from typhoid fever, any time after the first week. If the organism under consideration be typhoid its motion ceases, the cells becoming agglutinated, when they are seen in clumps or masses in the field of the microscope. If the organism is not typhoid the motion continues. The writer has tried the test upon a number of mobile organisms with the result of convincing himself that the antitoxin of typhoid fever does not act upon other mobile bacteria in the way that it does upon the germ to which it owes its origin.]

Wide variations exist in the total number of microörganisms present in sewage at different times and in different places, as might be predicted. Temperature is one of the

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most important factors in determining the rapidity of their reproduction and increase in numbers. Dilution of sewage by rainfalls exerts a marked modifying influence.

A striking difference exists between the organisms found in sewage air and in sewage itself. The molds which predominate in the former are very rarely observed in the latter. Out of the many thousand colonies which arose on numerous plates made from London sewage mold fungi occurred only seven times, and of these seven only one was allied to the common species existing in sewage air. These results coincide with those obtained by Jordan and others.

In showing the influence of locality on the number of bacteria present in different parts of a river the following observations are of value: The water of the Seine at Choisy, before reaching Paris, is found to contain 300 bacteria; at Bercy, 1,200; at St. Denis, after receiving sewer water from the city, 200,000 germs per cubic centimeter (Miquel). Water of the Spree beyond Kopenick, 82,000 bacteria. Two hundred steps below the mouth of the Panke, 940,000; below the mouth of the Panke, 1,800,000 (Koch). Water of the Main river above the city of Würtzburg, in the month of February, 520; below the city, 15,500 (Rosenburg). The water of the Thames in the autumn of 1885, in the vicinity of London bridge, two hours after high water, contained 45,000 germs per cubic centimeter; the water of the Lea at Lea bridge, 4,200,000 (Bischoff). The water of the Oder, collected within the limits of the city of Stettin, was found by Link to contain from 5,240 to 15,000 bacteria per cubic centimeter; that of the Limmat, at Zurich, 346 in one specimen and 508 in another (Cramer). The water of the Spree river of Berlin contains 400 bacteria at the Stralauer works.

Adametz (1888) has described 87 species of bacteria obtained by him from water in the vicinity of Vienna; Maschek found 55 different species in the drinking water used at Leitmeritz; and Tils (1890) has described 59 species obtained from the city water used at Freiburg.

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The following are the ordinary pathogenic organisms found in water :

Pathogenic Bacilli.—Bacillus typhi abdominalis (Eberth, Gaffky), *Bacillus erysipelatos suis* ("*Bacillus murisepticus*," Koch), *Bacillus septicæmiæ hæmorrhagicæ* ("*Bacillus cuniculicida*," Koch), *Proteus vulgaris* (Hauser), *Proteus mirabilis* (Hauser), *Bacillus canalis capsulatus* (Mori), *Bacillus canalis parvus* (Mori), *Spirillum choleræ Asiaticæ* (Comma bacillus, Koch), and a group of spirilla closely resembling it ; *Bacillus venenosus* (Vaughan), *Bacillus coli communis* (Escherich), *Bacillus hydrophilus fuscus* (Sanarelli), *Bacillus venenosus brevis* (Vaughan), *Bacillus venenosus invisibilis* (Vaughan), *Bacillus venenosus liquefaciens* (Vaughan).

Pathogenic Micrococci.—Staphylococcus pyogenes aureus (Rosenbach), Micrococcus of Heydenreich—"*Micrococcus Biskra.*" The former of the two last named germs being the common producer of abscesses, inflammations and phlegmons.

Concerning the probable presence of the *Plasmodium malaria* in drinking water that is charged with vegetable matter from low marshy districts, we have an important suggestion from the lower Mississippi Valley. So extensively did this disease prevail in the large tract—the delta—between the Mississippi and Yazoo rivers, population of the region seemed for a time to be impossible. Now the use of artesian wells there has brought a wonderful change. The residents of the delta used to drink the water from small surface streams, shallow wells and sluggish bayous. As a result of the use of water free from such surface contamination the region has been robbed of many of its terrors and has proved to be exceedingly healthy. For hundreds of years the Roman Campagna was the home of the deadly fever called Roman fever. The water-supply of the "Eternal City" was very poor and the fever made great ravages ; but since improvement in the water-supply the death-rate of Rome is lower than that of Naples, Florence, Turin and Milan, and there occurs scarcely a death in Rome from malarial disease con-

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tracted within the city. While we in the North do not have the extensive swamps of the Mississippi region, still there is in the above an important lesson for us.

The following table may here be of interest:

Name of City.	Total Water Revenue.	Annual Receipts from Mineral Water.	Average Daily Consumption in Gallons.	Revenue per Million Gals.	Meter rate per 1000 Gals.
New York . .	$3,457,347.04	$1,520,897.20	154,000,000	$ 61.50	.13⅓
Chicago . . .	2,592,111.67	795,292.67	174,000,000	40.81	.08–.10
Philadelphia .	2,634,481.52	125,705.73	163,800,000	44.06	.08
Brooklyn . . .	1,486,003.38	295,087.57	58,054,000	70.12	.10–.15
St. Louis . . .	1,235,933.30	555,850.50	44,162,000	76.67	.10–.30
Boston	1,838,494.30	587,850.50	46,740,000	107.76	.16–.18⅔
Baltimore . .	869,777.67	159,459.86	47,000,000	50.70	.06
Cincinnati . .	988,053.90	188,034.05	42,119,000	64.27	.09–.15
Cleveland . .	598,433.50	258,885.09	36,443,000	44.98	.05⅓

Thus it will be seen that the subject of the water-supply in every large city is one that involves great financial considerations, and it would appear consistent to expend a little more (or, in some instances, perhaps less,) and improve the *quality* of the supply.

As I have discussed at length the subject of the purification of water for drinking purposes, in the January, 1897, number of *The Chautauquan*, I will not touch further upon it here.

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ON THE APPARENT STRUCTURE OF THE SCALES OF SEIRA BUSKII IN RELATION TO THE SCALES OF LEPIDOCYRTUS CURVICOLLIS.

R. L. MADDOX, M. D.,* Hon. F. R. M. S., Southampton, England.

The structure of the scale of *Lepidocyrtus curvicollis* has occupied the attention of microscopists from the time it was adopted by opticians in England, and I believe also in America, as a test object whereby to ascertain the correction of their objectives, and yet the question may be asked, though so often examined, has its structure been satisfactorily established or accepted? Would not the general answer be a negative one? It is feared it would. Now, I am not bold enough to do more than to put in a plea for the careful examination of the scale of *Seira buskii*, which is more robust, in the hope it may help in the elucidation of the finer marked scale of *Curvicollis*.

It may be remarked that both scales are derived from the Poduridæ, and as they both present the markings termed "notes of exclamation," may we not reasonably consider that both are constructed upon the same plan, differing only in minuteness of detail? I have been led to this subject by finding some appearances in the scales of *Buskii*, which seemed to me to offer a chance of getting a little insight into the real structure of both scales; but I at once readily confess still to a difficulty in formulating a theory or statement not open to question. It is, therefore, under this difficulty the subject is approached and the following remarks offered for the consideration of the members of your society. There is, however, one point on which I have less hesitation, viz.,

* Elected an honorary member of the American Microscopical Society, August 20, 1896.

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that the appearances the scale of *Seira* have presented, prevent participating in the supposition that the notes in either are due to minute spinous projections on either the upper or under surface of the scale, or that they are anything more than optically due to refraction through adherent and separated portions of the membranes forming the surfaces and the inner framework of the scale, surrounded by a more highly refracting substance.

As the appearances in *Curvicollis* are so well known, let me turn at once to the scales of *Buskii*, where the notes are so much larger. On taking a general view of these scales, we find they differ very much in size and in the robustness of the notes on the scale. The small scales often have the notes stronger and thicker, or wider than in the large scales, and in some the center ones run without a break from the stem to nearly the point. This is shown in the Photograph No. 1, × 710 diameters; moreover, these small scales often scarcely show any light areas in the note of exclamation, consequently it seems to present a more or less solid structure. While the larger scales have these notes many times more numerous than the smaller scales, and almost all of them under high powers show long open or light central areas, as in Photograph No. 2, × 930 diameters. Looking on these as types of the perfect scale, we meet with others which show that there is between these notes throughout the scale a more highly refracting substance, which does not in *Buskii* appear to be of such a fatty nature as in *Curvicollis*. This substance in many of the scales on the slide, which is an old one, has receded in continuity from the stem toward the point, appearing abruptly broken across in the interspaces between the notes, yet at the same time leaving the lower point or tail of the note a mere line ridge, or bar starting from the base or stem end of the scale. This is seen in Photograph No. 3, × 710 diameters. A step further, and this material is found in a granular condition, occupying the interspaces between the lower notes while the notes remain nearly perfect toward the point of the scale. See Photograph No. 4,

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× 1000, and Photograph No. 3, × 710, where the process is fainter. One step more, and now this substance is seen dispersed throughout the scale in one uniform mass, save at minute points on the edges, and only darkish lines, bars or spaces are seen more or less extending from the stem to the point. Here we may say we have entire obliteration of the notes. What has become of the notes? They have vanished, leaving only shaded areas in their place. This appearance is shown in Photograph No. 5, × 1000 diameters. It was then finding these scales that led me to hope some clue could be gathered as to the cause of the notes of exclamation, and induced me to puzzle over what might be the entire structure of the scale. For comparison I had by me the large engraved plate of the scale of *Lepisma sacharina* with copious marginal notes, given me November, 1863, by the distinguished microscopist and draughtsman, the late Richard Beck. Mr. Beck found in his scale two sets of bars or lines which intersecting each other at different angles thus produced either vertical or oblique notes "by the refraction of light, when the structure of the outer and inner surfaces of the scale cross each other in different directions." And he states "that when moisture is on the outer surface, the radiating or the oblique lines become continuous, but when the moisture is on the inner surface, then the longitudinal markings are continuous, and when no fluid is present, both sets of markings are interrupted." According to this view, the obliteration of the notes is the effect of moisture. Now, in the scales of *Buskii*, it seemed to me, the obliteration of the notes is due to the refraction, breaking up or universal spreading of the inclosed more highly refracting substance; in other words, that it is broken down and no longer lies only in the interspaces between the notes. At the edges right and left of the base of some of the scales, we have more or less indications of the oblique lines found in *Lepisma*, but I have failed to trace them, and if they exist beyond or towards the stem or the central part of the scale, I consider they do not from their direction any way share in the cause of the long or longitudi-

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nal notes in *Buskii*. How then can the appearance of these notes be explained? May we not suppose that the structure of the entire scale consists of a very delicate oblong bag, more or less conversely curved on its outer surface, and more or less flat or concave on its inner surface, or in cross section somewhat plano-convex or convexo-concave, and that these delicate membranes are supported or strengthened by an inner framework of longitudinal bars, extending from the base and gradually approaching the center, until they nearly or quite meet at the tip of the scale, all but the most central ones taking a slight curve right and left at the base, and following the shape of the scale. The membranes of the bag I regard as plain, though some may possess a few lines or bars radiating from the stem each side, which I regard as due to curved notes embracing the lower rounded or more inflated edges of the scale. This small sac seems filled with a semi-solid or thick fluid substance of a rather higher refracting power than either the membrane or framework. In addition, if we suppose that the two membranes, *i. e.*, the upper and under, adhere in part to the longitudinal bars of the supporting inner frame in their course to the point of the scale, and break away from the bars at various points to again unite either to the same bar or its neighbor, repeating the process of adherence or close approximation and separation all over the scale, should we not have the bright areas; and if the bars were themselves at all concave and the membranes touched the edges, but not the center for the whole length of the note, should we not have the appearance of the open areas, the head of the note being closed in by the capillarity of the semi-fluid interspace substance? This would tend to give the bars a more or less double outline, as in the Photograph No. 6, × 930 diameters. This may appear as only a fanciful rendering of the structure, but had I to try and fabricate a monster scale with any transparent plastic substance, it would be the first method adopted. The point or dark tail of the note would have the bar and membranes closely adherent, the clear areas the membranes only adherent

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to the edges of the bar, and then interrupted to repeat this arrangement. It may be noted that where the scale commences to have the notes changed into the appearance of only longitudinal lines or bars, the change always begins, as far as I can find, at the stem end of the scale, though the breaking up of the inner substance into a granular state may be only scantily apparent, and this change is regarded as sufficient to disturb the adherence of the membranes along the bars, working the same change gradually toward the apex, as seen in Photograph No. 4, × 1000 diameters. If complete, then the appearance shown in Photograph No. 5, at the same magnification, though the framework is only faintly indicated, possibly due to the inner substance having lost its original character and refractive quality. It may be said there is no true framework, and the bars are formed by folds or creases in the upper or under membranes; but in my examination and dissection, years since, extending over a long period, I was able to find an inner framework in the butterfly scale, and this tempts me to the idea of an inner support in the scale of *Buskii*.

To repeat, the dark line in the tail of the note is suggested as caused by the close adherence there of the three parts, upper and under membrane with that part of the frame and as it exists for a variable distance, so the tail of the note may be of variable lengths. The open or central light area is regarded as one of the two membranes adhering only at the edges, right and left of that bar, the interspaced material being drawn by capillarity close to the edges, thus assisting in forming the somewhat sharp outline at that part, and this process to continue through the length of the bar to the point of the scale, or to occur in the neighboring bars irregularly in *Buskii*, but more regularly in *Curvicollis*, where the interspace material is more or less fluid, and of a more refractive nature. The photograph of the Podura scale *Curvicollis*, is given just to show that in certain positions in relation to the illuminating rays, the indication of lines becomes apparent, and these are often lost in injury to the scale by reason of its

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delicacy and the highly refracting quality of the interspace material.

It must be distinctly understood that the above remarks are not put forward in any dogmatic spirit, but in the hope that in directing attention to the scale of *Buskii*, clearer views may be formulated, both of its structure and that of *Curvicollis*. At any rate it is trusted that the subject may prove of some interest to the members of your Society, and the readers of your TRANSACTIONS which abounds in interesting details connected with microscopy.

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No. 1. A small perfect scale, × 710 diameters.

No. 2. The same large scale as No. 7, showing the notes, × 930 diameters.

No. 3. A medium scale, showing the bars at the base, the interspace material receding and granulation begun, × 710 diameters.

No. 4. A medium scale, with the granulation further advanced towards the apex, × 1000 diameters.

No 5. A medium scale, with the change completed and the notes lost, leaving only faint indications of previous existence, × 1000 diameters.

No. 6. The same large scale as Nos. 2 and 7, focused into showing a double line appearance, × 930 diameters.

No. 7. A large perfect scale, × 668 diameters.

No. 8. A *Curvicollis* scale, showing a sort of lined appearance, especially on the right hand, and by way of comparison.

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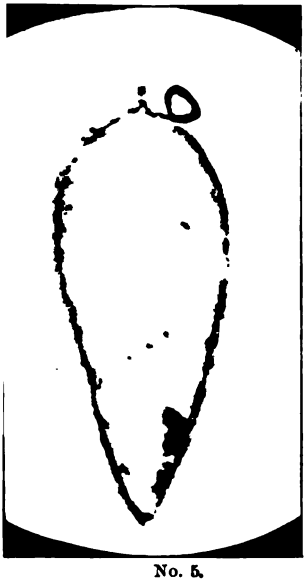
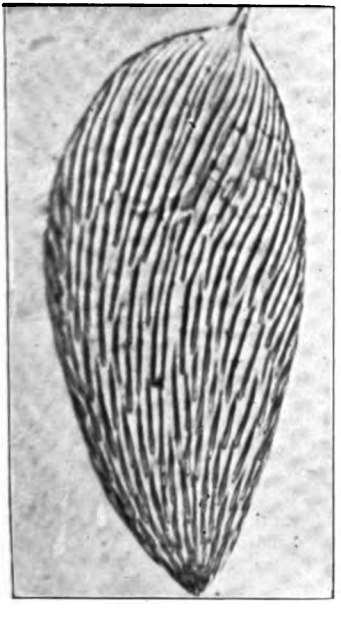


No. 5.

No. 3.

No. 4.



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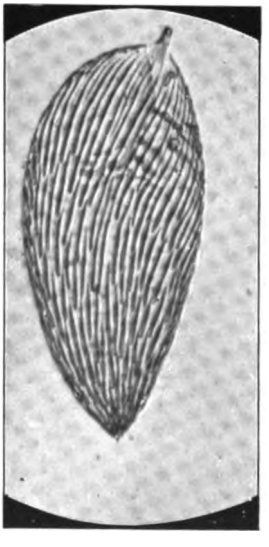

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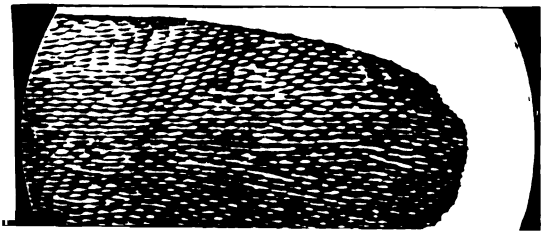

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THE RED BLOOD CORPUSCLE IN LEGAL MEDICINE.

MOSES C. WHITE, M. D., NEW HAVEN, CONN.

Much has been written on this subject, and Clark Bell, Esq., in an able symposium, has shown that the consensus of scientific opinion may be summarised in the following declaration, viz., that "by careful and competent observers, with instruments of high power, a reliable discrimination can be made between human blood and the blood of other mammals, when the size of the red corpuscle is much smaller than those of man, notably in the case of the ox, the horse, the goat, the sheep, the pig and most other mammals."

In contravention of this opinion, Professor Marshall D. Ewell, M. D., in his address delivered at Rochester, N. Y., in August, 1892,* advances the opinion that "the very utmost the present state of science enables us to state is that the blood in question is or is not that of a mammal, a bird, fish or reptile. To say more than this is, in my judgment, without warrant to imperil human life, and, in the words of the late Dr. Woodward, to make scientific experts more dangerous to society than the very criminals they are called upon to convict."

As this startling proposition has never been answered in the TRANSACTIONS of the American Microscopical Society, I offer the following discussion of the subject, which I had the honor to read before the Medico-Legal Society in December, 1894. Professor Ewell's arguments, which I controvert, will be found in the TRANSACTIONS of this society and in the *Medico-Legal Journal*, Vol. X., No. 2, September, 1892.

Professor Ewell ruled a series of eleven lines on glass, giving ten spaces of approximately 0.004 inch, and another

* TRANSACTIONS American Microscopical Society, Vol. XIV.

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set with five spaces about 0.008 inch, and gave these plates to several microscopists to report from careful measurements the values of the ruled spaces. According to Professor Ewell, in the results of the measurements made by the six microscopists, there were differences in the results of their measurements greater than $\frac{1}{9000}$ of an inch, which Professor Ewell says is greater than the greatest difference between the different measurements of the blood corpuscles of man and some common domestic animals, *i. e.*, dogs. Although these measurements were made with low powers, 1 in., $\frac{3}{5}$ in., $\frac{1}{2}$ in., $\frac{4}{10}$ in., $\frac{1}{4}$ in., and $\frac{1}{8}$ in. objectives, giving magnifying powers approximately of 100 to 500 diameters. Professor Ewell appears to think such powers might give as accurate measurements as higher powers. He says:

> To the objection that a low power was used in every instance, it may be answered that where the power is high enough to make the object clearly visible and of an appreciable size, as was the case in these measurements, and where a series of measurements are made, the result is practically the same as if a higher power were used.

The consequences of this startling proposition are too great to allow it to be passed over without careful criticism.

Gulliver's measurements of blood corpuscles were made with a magnifying power of 600 diameters. Woodward, Richardson, Treadwell, Wormley, Piper, Formad and most microscopists who figure in Medico-Legal cases at the present day, have used powers ranging from 1,000 to 2,500 or 3,000 diameters. Professor Ewell, himself, favors 1,000 diameters for use in measuring blood corpuscles, and yet he puts forth the discrepancies between measurements of different observers with powers of 100 to 250 diameters to discredit the distinctions observed with powers of 1,000 diameters, or even 3,000 diameters between the blood corpuscles of man and the domestic animals.

As a further answer to Professor Ewell we may cite observations recorded by Professor Wormley, in Micro-Chemistry of Poisons, page 728, wherein a series of ten spaces ruled on glass, measured by three observers with different instruments,

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the results did not differ more than $\frac{1}{200000}$ of an inch. Also two independent series of measurements with high powers, of twenty designated blood corpuscles were absolutely identical for sixteen corpuscles, and for the other four the greatest difference was only $\frac{1}{200000}$ of an inch. Still further, Professor Wormley reports the measurement of seven human blood corpuscles with powers varying from 1,150 to 3,500 diameters by different micrometers, and by drawing with the camera lucida. The range of averages of the different measurements was $\frac{1}{3224}$ of an inch, to $\frac{1}{3275}$ of an inch, as measured by the camera lucida. The mean of the averages being $\frac{1}{3250}$ of an inch. The same seven corpuscles measured by Dr. Richardson, using a cobweb micrometer, gave a final average of $\frac{1}{3246}$ of an inch, differing from the former average by something less than $\frac{1}{350000}$ of an inch.

To further show the uniformity of results obtained by different observers, I would mention that on a slide of human blood irregularly spread and dried so that the corpuscles on one-half of the slide appeared larger than on the other part, Dr. T. measured, with high powers, seventy-five corpuscles on the side showing the larger, and seventy-five on the side showing the smaller corpuscles. The average of the 150 corpuscles thus measured was $\frac{1}{3667}$ of an inch. The writer using a power of 1,400 diameters and a cobweb micrometer measured fifty corpuscles on one part of the same slide and fifty on the opposite part of the slide, and found the average of the 100 corpuscles to be $\frac{1}{3665}$ of an inch, differing by only $\frac{1}{67119777}$ of an inch from the results obtained by Dr. T. on the same slide.

With the above demonstrations of corresponding results of measurements made by the microscope in the hands of independent observers, using powers of 1,000 diameters and upward, I think I may dismiss the objections of Professor Ewell, based on slight differences obtained by different observers with powers ranging from 100 to 400 diameters, whether due to defects of instruments or to the personal equation of the observers.

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Boston, with a $\frac{1}{75}$ inch objective, made by R. B. Tolles, and a Jackson eye-piece micrometer made by Tolles, ruled by Professor Rogers, and a stage micrometer, ruled, tested and rated by Professor Rogers:

SOURCE OF BLOOD. Measurement in *Mikrons*. By J. B. TREADWELL, M. D.*	No. of Discs.	Mean Diam.	Max. Min.	Av. by 10's.	Av. by 20's.	Av. by 50's.	Av. by 100's.	Av. by 200's.
Five men, ages 23 to 49 years.	1000	7.941	5.773	7.697	7.782	7 845	7.884	7.903
			9 394	8.152	8.110	8 061	8.046	7.983
Five women, ages 18 to 55 years	1000	7.927	6.350	7.825	7.787	7 873	7 901	7.913
			9 287	8.152	8.026	7.993	7 963	7.950
Three infants at birth, 1 male, 2 females	600	7.950	4 233	7 667	7 716	7.833	7.918	7 938
			10.160	8.298	8.100	8.031	7.983	7.970
Boy, 8 years old	200	7.983	6.929	7.747	7.828	7.891	7.965	7.983
			9.160	8 284	8.191	8 079	8.000	
Man, 70 years old	200	7.916	7.005	7.658	7.662	7.768	7 852	7.916
			9.236	8.121	8.105	8.028	7.980	
Fifteen persons, as above	3000	7.938	4 233	7.658	7.662	7.768	7.852	7.903
			10.160	8.298	8.191	8.079	8.046	7.983
Blood stains (human) restored	1000	7.910	5 570	7.700	7.723			
			9 687	8.189	8.010			
Woman, 19 years old, anemia	100	7.346	3.464	6.674	7.012	7.177	7.346	
			9.237	7.605	7.628	7.515		
Child, 6 weeks old, starved to death	100	7.573	4.465	7.328	7 493	7.574	7.573	
			8.636	7.712	7.639	7.574		
Twenty-five dogs, each 100 corpuscles	2500	6 918	4.618	6.138	6 445	6.523	6.673	
			8.931	7.352	7 305	7.258	7.198	
Pigs, 2 of 3 months, 1 of 2 weeks	600	6.101	3.849	5.418	5.757	5 880	6.028	6.069
			8.391	6.520	6 466	6.246	6.169	6.144
Ox, 3 males, 2 females; 1 of 1 day, 1 of 3 months	1000	5.436	3.916	5 150	5.296	5.345	5.347	5.406
			6.774	5.703	5.622	5.543	5.482	5.461
Horse, 10 years old	200	5.503	4.618	5.203	5.257	5 473	5.493	
			6.774	5.818	5.633	5.553	5.513	
Ass, 1 male, 1 female	400	6.293	5.003	6.018	6.138	6.201	6.219	6.259
			7.697	6.566	6.539	6.377	6.376	6.326
Mule, 6 years old	200	5.421	3.464	5.280	5.327	5.377	5.419	
			6.312	5.680	5.545	5 472	5.424	
Two cats, 1 adult, 1 kitten 3 weeks old	400	5.463	2.617	4.311	4.361	5.268	5.419	5 431
			6.774	5 780	5.676	5.573	5 522	5.495
Male cat, stupefied 5 hours by alcohol	100	5.489	4 772	5.327	5.426	5.479	5.489	
			7.235	5.680	5 846	5 498		
Guinea-pig, male, 3 months	200	7.476	5.849	7.231	7.309	7.249	7.393	
			8.390	8.698	7.659	7.634	7.559	
Two rabbits, 1 white, 1 mixed	400	6.365	4.618	6 196	6.227	6.294	6.349	6.354
			8.082	6.596	6.845	6.409	6.383	6.377
Rat, male	200	6.500	3.079	6.289	6 346	6.441	6.490	
			8.005	6.820	6.695	6.694	6.510	
Sheep, male, 15 days, female, 1 year	400	4.745	3.079	4.503	4.588	4.665	4.725	4.744
			6.774	4.972	4.869	4.785	4.764	4.746
Goat, 1 male, 1 female	400	3.567	2.617	3.394	3.401	3.467	3.535	3 546
			4.080	4.764	3.710	3.693	3.638	3.587

* From an article on Blood Stains by M. C. White, M. D, in *Wood's Handbook of Medicine*.

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In this table the measurement of 3,000 corpuscles from man are given, 200 having been taken from each of fifteen different persons. The measurements are given, maximum and minimum, by tens, by twenties, by fifties, by hundreds and by two hundreds. Six hundred from pigs were measured; 200 from a pig three weeks old, 200 from one of two months, and 200 from a pig three months old.

As the corpuscles of the pig have a larger average than those of the ox, horse, sheep or goat, we make our comparisons between the blood of man and the blood of these three young pigs.

	Mikrons.		Mikrons.
Man, smallest ten	7.658	Largest ten	8 298
Pig, " "	5.418	" "	6.520
Man, smallest twenty	7.662	Largest twenty	8.191
Pig, " "	5.757	" "	6.466
Man, smallest fifty	7.768	Largest fifty	8.079
Pig, " "	5.880	" "	6.240
Man, smallest hundred . .	7.852	Largest hundred	8.046
Pig, " " . . .	6.028	" "	6.169

Smallest ten from man . . . . . 7,658 mikrons.
Largest ten from the pig . . . . . 6,520 "

Difference . . . . . 1,128 mikrons.
This equals 0.000045 of an inch, — $\frac{1}{22222}$ inch.

Smallest twenty from man . . . . . 7.662 mikrons.
Largest twenty from the pig . . . . . 6,466 "

Difference . . . . . 1,196 mikrons.
This equals 0.000047 of an inch, — $\frac{1}{21276}$ inch.

Smallest fifty from man . . . . . 7,768 mikrons.
Largest fifty from the pig . . . . . 6,246 "

Difference . . . . . 1,522 mikrons.
This equals 0.000060 of an inch, — $\frac{1}{16666}$ inch.

Smallest hundred from man . . . . 7,852 mikrons.
Largest hundred from the pig . . . . 6,169 "

Difference . . . . . 1,683 mikrons.
This equals 0.000066 of an inch, — $\frac{1}{15151}$ inch.

We thus see that where only ten corpuscles are measured from man, taking the smallest average of ten consecutively measured, this average for an adult man is one-sixth larger than the largest average of ten consecutively measured from

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a young pig only three weeks old; while the smallest average of one hundred from man taken consecutively is between one-fifth and one-fourth larger than the largest one hundred taken from the pig.

TABLE SHOWING DISTRIBUTION OF VARIOUS SIZES OF BLOOD-CORPUSCLES MEASURED IN PARTS OF AN INCH. (BY J. B. TREADWELL, M. D.*)

Fractions of an inch	Man.	Dog.	Pig.	Ox.	Sheep.	Goat.
1-2700	2					
1-2800	6					
1-2900	14					
1-3000	32					
1-3100	33					
1-3200	46	6				
1-3300	37	17				
1-3400	14	23	2			
1-3500	9	24	2			
1-3600	4	58	4			
1-3700	1	23	17			
1-3800	2	12	21	1		
1-3900		10	9	3		
1-4000		9	31	1		
1-4100		12	34	22		
1-4200		3	15	16		
1-4300		1	7	7		
1-4400		2	11	10		
1-4500			9	17		
1-4600			8	15	2	
1-4700			18	31	15	
1-4800			4	15	8	
1-4900			4	1	6	
1-5000			1	9	19	
1-5100			2	8	11	
1-5200			0	3	20	
1-5300			0	5	17	
1-5400			0	3	30	
1-5500			1	13	47	
1-5600					11	
1-5700					3	
1-5800					6	3
1-5900					1	0
1-6000					0	2
1-6100					0	2
1-6200					0	4
1-6300					2	5
1-6400					1	30
1-6500					0	0
1-6600					1	45
1-6700						20
1-6800						29
1-6900						0
1-7000						15
1-7100						17
1-7200						0
1-7300						15
1-7400						0
1-7500						4
1-7600						4
1-7700						0
1-7800						1
1-7900						0
1-8000						4
No. of corpuscles	200	200	200	200	200	200
Maximum	1-2727	1-3203	1-3473	1-3837	1-4647	1-5892
Minimum	1-3870	1-4400	1-5500	1-5500	1-6600	1-8048
Mean	1-3197	1-3657	1-4227	1-4654	1-6204	1-6839

* The number of corpuscles include all found between the fraction opposite to which they are placed and the next succeeding fraction of an inch.

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Professor Ewell says—*Medico-Legal Journal*, Vol. X., page 201—that "fasting diminishes both the size and number of the red blood corpuscles. This is also true of various drugs." This agrees with my case of a child six months old, starved to death, given in the preceding table.

A stain from human blood in such a case might possibly be mistaken for that of a dog or a pig. Such a condition might tend to acquit a guilty person, but never to convict of murder an innocent man.

Professor Ewell says: "Many diseases alter the size of the red blood corpuscles," and he gives the result of careful measurements which he has made in a number of diseases. I take great pleasure in acknowledging the excellent work done in this line by Professor Ewell, and, for the present discussion, I have compiled from Professor Ewell's reports the following table:

Source of Blood.	No. of corpuscles.	Mean in Mikrons.	Max.	Min.	By 100's Max.	By 100's Min.
Robust Man	650	8.03	9.98	5.03	8.28	7.95
Boy, 36 hours old	200	8.86	11.39	5.70	9.06	8.65
Adult Man	100	7.85	9 32	6.73		
Purpura Hemorrhagica	200	8.26	10.87	3.45	8.28	8.25
Two cases Pseudo Leucocythemia	400	8.04	11.04	6.56	8.55	8.42
Tuberculosis Anemic	100	8.35	10.70	5 35		
Plumbism	100	8.65	10.10	5.18		
Gastritis?	100	8 32	10.18	6 22		
Two cases Syphilis	200	8.11	9.32	3.97	8.11	8 11
Erysipelas	100	7.83	9.15	6.90		
Pernicious Anemia	100	7.69	9 93	6.04		
Menstrual blood	100	7.71	8.80	5.76		
	2,350	8.14	11.39	3.45	9.06	7.95

If we take $\frac{1}{3200}$ of an inch, or 7.937 mikrons as the generally accepted average diameter of human blood corpuscles, and compare it with the average obtained by Professor Ewell from blood in diseases, we find his largest average is in plumbism or lead poisoning and is 8.65 mikrons, $\frac{10}{113}$ or a little over one-twelfth part larger than the normal average.

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Now, if we take the young pig, which, excepting the dog, is the domestic animal having the largest corpuscles, and to the normal average of the pig, 6.10 mikrons, add $\frac{10}{113}$ its diameter, 0.55 mikrons, we shall obtain for the blood of a diseased pig an average diameter of 6.65, which is smaller by 1.287 mikrons than the average human blood corpuscle.

This possible enlargement by disease is only one-third the difference between the blood corpuscle of man and that of the pig.

Certainly no microscopist of ordinary skill, provided with modern instruments, rated to measure $\frac{1}{1000000}$ of an inch, like the ordinary filar micrometer, with powers of 1,000 or 1,500 diameter; or even the glass eye-piece micrometer, rated to $\frac{1}{100000}$ or even $\frac{1}{50000}$ of an inch, would be in any danger of mistaking blood of even a young pig (whose corpuscles had been enlarged by disease as much as that of the man suffering by lead poisoning) for human blood."

Professor Ewell further objects that "drying the blood corpuscles in a clot multiplies the difficulty of identification," and says: "It has never been proven that dry corpuscles can be restored to their normal proportions," and continuing says: "In view of the foregoing it is impossible in the present state of science to say of a given specimen of blood, fresh or dry, more than that it is (or is not) the blood of a mammal."—*Medico-Legal Journal*, Vol. X., p. 201.

In answer to this objection, I again refer to the table of measurements given above, which I have quoted in part from my article on Blood Stains in *Wood's Handbook*, showing measurements of 3,000 corpuscles of fresh human blood, including males and females of all ages, from the infant at birth to the man of seventy, contrasted with 1,000 corpuscles restored from stains of human blood. The measurements are in mikrons.

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Number of Individuals.	Number of corpuscles.	Average.	Max. Min.	By 10's.	By 20's.	By 50's.	By 100's.	By 200's.
Fifteen persons, as stated	3,000	7.938	4 233 10.160	7.658 8.298	7.662 8.191	7.768 8.079	7.852 8.046	7.913 7.983
Blood stains—human	1,000	7.910	5.570 9.687	7.700 8.184	7.723 8.010			
Three young pigs	600	6.101	3.849 8.391	5 418 6.520	5.757 6.466	5.880 6.246	6.028 6 169	6.069 6.144

Here we see that the average of 1,000 corpuscles restored and measured from stains of human blood is 7.910 mikrons, all probably being stains from the blood of adults; while the average from fifteen persons (three being infants) is 7.938 mikrons, the maximum and minimum, where infants are included, in fresh blood being a little more widely separated than in the restored stains from adults. So also the range, when taken by tens or twenties, is a little less for restored blood than for fresh blood, for the same reason. No closer similarity of results could have been expected had all the measurements been taken from fresh blood, one set including infants and the other taken from adults alone. The fact that there is this difference given above goes to show the great accuracy and perfect restoration of dried blood corpuscles to their normal dimensions.

As a further confirmation of the possibility of restoring dried blood stains to a condition in which their dimensions can be properly compared with fresh blood corpuscles, we copy, by permission, the following table from Professor Wormley's Micro-Chemistry of Poisons:

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EXAMINATION OF OLD BLOOD STAINS.

Animal.	Age of Stain.	Remarks.	Average.	Fresh Blood.
(1) Human .	2 months old,	Stain unknown	$\frac{1}{3350}$ in.	$\frac{1}{3150}$ in.
(2) " .	2½ " "	Stain	$\frac{1}{3336}$ in.	$\frac{1}{3150}$ in.
(3) " .	3 " "	"	$\frac{1}{3334}$ in.	$\frac{1}{3150}$ in.
(4) " .	19 " "	Clot	$\frac{1}{3350}$ in.	$\frac{1}{3150}$ in.
(5) Elephant,	13 " "	"	$\frac{1}{3626}$ in.	$\frac{1}{3731}$ in.
(6) Dog . . .	4 " "	Trace of stain unknown,	$\frac{1}{3636}$ in.	$\frac{1}{3561}$ in.
(7) Rabbit . .	18 " "	Clot	$\frac{1}{3633}$ in.	$\frac{1}{3633}$ in.
(8) Ox . . .	16 " "	Stain	$\frac{1}{4444}$ in.	$\frac{1}{4319}$ in.
(9) " . . .	32 " "	Stain unknown	$\frac{1}{4436}$ in.	$\frac{1}{4319}$ in.
(10) " . . .	4½ years old .	Clot	$\frac{1}{4336}$ in.	$\frac{1}{4319}$ in.
(11) Buffalo .	18 months old .	"	$\frac{1}{4318}$ in.	$\frac{1}{4361}$ in.
(12) Goat . . .	17 " " .	Stain	$\frac{1}{3357}$ in.	$\frac{1}{3135}$ in.
(13) Ibex . . .	18 " "	Clot	$\frac{1}{3678}$ in.	$\frac{1}{3444}$ in.

In the case of the human blood No. 1, two months old, the deposit was in the form of a thin stain on muslin, and its nature, other than that it was mammalian blood, was unknown at the time of examination. The corpuscles were readily found, and two series of thirty corpuscles were measured. In the human blood two and a half months old, fifty corpuscles, ranging from $\frac{1}{3125}$ to $\frac{1}{3448}$ of an inch were measured.

The blood stain of the dog, No. 6, was prepared by Dr. Frankenberg, and consisted of a single stain so minute as to be barely visible to the naked eye; its nature at the time of the examination was unknown. In the ox blood four and a half years old, the corpuscles were readily obtained, and two closely concordant series of measurements were made.

The table of Professor Formad, Collective Results of Some of the Series of Measurements of Red Blood Corpuscles in Blood Stains, in his article on Mammalian blood (*Journal of Medicine and Surgery*, July, 1888, copied in the *Medico-Legal Journal*, Vol. X., p. 157), proves still further the successful recovery and measurement of red corpuscles from dried blood stains.

These tables show that blood corpuscles can be, and have been, recovered and measured and found to correspond with the average of fresh blood of the same animal from which the stain was taken as closely as the corpuscles of any two animals of the same species correspond when fresh.

The only remaining possibility of mistaking stains from blood of a pig, ox, horse, sheep or goat for human blood,

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when fifty or one hundred corpuscles have been measured and found to average 7.9 mikrons, or even 7.0 mikrons, is to suppose that the blood corpuscles have become larger than fresh blood, or that the expert has *wickedly* and *purposely* measured only the largest corpuscles and has not done honest work.

Professor Ewell says that "by selecting the corpuscles it would be possible for a dishonest observer to make the average much larger or smaller than the above given (in his table) without the possibility of detection—a fact the bearing of which upon the value of expert testimony upon this subject is so obvious as to need no comment." We have never claimed, and shall not now claim, that it is any more difficult for a witness to swear falsely in regard to blood stains than in regard to any other subject. But it would be quite possible on cross examination to ask the expert whether in measuring the corpuscles obtained from a stain he had measured all the well-defined corpuscles in the field of the microscope, or whether he had selected the large corpuscles and rejected others which were smaller.

In the case of State of Connecticut vs. Herbert H. Hayden, Superior Court Special, October term, 1879, Dr. J. J. Woodward, U. S. A., gave testimony for the defense in regard to examination of blood stains.* He stated that in 1875 he had published in the *American Journal of Medical Science* the measurements of thirteen sets of human blood corpuscles, about fifty in each set. The average diameter of the whole series was 0.000295 inch. The largest set of fifty corpuscles measured 0.000304 inch, and the smallest set 0.000295 inch, but that he left out some of the larger corpuscles because they were not perfectly circular, and that the result would give an average rather too small for the general average of human blood.

That he also measured nine sets of about fifty in a set from the blood of a dog; the average of the nine sets from the dog was 0.000290 inch. Some of the averages of fifty from the dog were larger than some of the averages from men,

* Report of Court Stenographer printed by Black & Bryant, Boston, Mass.

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though the average from all dogs would be smaller than the average from all men. These were measured with an eye-piece micrometer ruled on glass.

In 1876 he published in the Proceedings of the American Medical Association measurements of twenty-two sets of corpuscles from men and thirteen sets from one dog. The average of all the sets from men, then published, was 0.000323 inch, which is larger than Gulliver's measurement of $\frac{1}{3200}$ inch by eleven millionths of an inch, but some of his averages of 150 corpuscles from a dog, published at that time, were also larger than some of his averages of 150 in a set from men.

All the measurements above referred to, published in 1876, were measured on photographic negatives.

Dr. Woodward also stated in the same case:

Within the last two or three weeks I have made a still further series of measurements of man and dog. I used for the purpose a cobweb micrometer, and the lens which of all other lenses in my acquaintance defines the best—namely, one of the Zeiss lenses; Zeiss of Jena.

With that lens and with a cobweb micrometer I made a series of measurements of forty corpuscles of human blood—it was from one of the assistants in the museum. I brought the corpuscle into the center of the field and I measured it with the cobweb micrometer, that is by turning the screw I set the cobweb on the extreme limit of the corpuscle so that the cobweb line just touched the edge on the outside, and then the readings of the turns of the screw gave the number of divisions.

I got an average of those forty corpuscles of 327 millionths of an inch—This was fifteen millionths of an inch larger than $\frac{1}{3300}$ of an inch. Now, in these 40 corpuscles, the biggest individual was 366 millionths of an inch.

Then I pricked my own finger and took a drop of my own blood and I measured fifty corpuscles, and there I got the average size to be 324 millionths of an inch, that you see is three millionths of an inch smaller than I just got on another set.

Then in another sample of human blood I got the figure 316 millionths of an inch on the measurements of twenty corpuscles. This last figure would differ only four millionths of an inch from Gulliver's $\frac{1}{3300}$ of an inch.

I measured, on the 14th of December of the present month, fifty corpuscles of the blood of a dog; it was a Newfoundland crossed with setter; a pup four weeks old. I measured fifty corpuscles, and the average was 326 millionths of an inch.

Q. Differing, I see, only one millionth of an inch from the first forty corpuscles of human blood?

A. Yes, and larger than my second fifty, larger than any of that set of human measurements, except the first, and larger than any of the photographic

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measurements I have published of dogs' blood except one. Well, then I measured another dog's blood on the 12th of December, a Scotch terrier, forty corpuscles, and I got an average of 320 millionths of an inch.

Q. That, I see, is larger than the last for human blood?

A. It is larger than the last for human blood, and larger than the $\frac{1}{3100}$ of Gulliver.

Q. It is larger by eight millionths than Gulliver's average?

A. Yes, sir; then also on the 14th of December I measured another dog's blood, a Gordon setter, full grown, and his blood was smaller than either of the young dogs I have spoken of, the average of twenty-nine corpuscles was 300 millionths of an inch.

Q. Excuse me for asking you how much larger that is than your figures published in 1875, of human blood?

A. It is five millionths of an inch larger.

Q. Then, if I understand it, you measured several other dogs at this time?

A. Not at this time, those were all I measured at this time, and I desire to put myself on record as distinctly denying that those measurements of the dog's blood would represent what you would be likely habitually to get if without picking out young dogs, and without selecting a spot to measure appearing to have unusually large corpuscles you were to measure dog's blood. You would then habitually get smaller figures than I have given here. The averages of the corpuscles of dogs would habitually be smaller than I have given here, but I have purposely selected for fixed measurements young dogs, for I knew they had bigger corpuscles, and, in the second place, in taking an old dog, I purposely selected a place (on the slide) where, to my trained eye, I saw there was a group of big corpuscles; that is the place on the thing I was measuring. My reason for doing that is to show that if you pick up a half dozen corpuscles at chance, you may chance on these big things. But I have nowhere stated, on the contrary I would like to read my express statement from my printed utterance, that the general average for the dog, for a great number of measurements, will be less than for a great number of men.

Q. You have stated that your object in selecting these dogs, and these large corpuscles of these dogs, was to show how it might innocently happen that in the measurements of a given number of corpuscles, say twenty, or thirty, or forty, or fifty, as you have here, you might get this large average?

A. Yes, and you would get it if you stumbled upon such a group.

In relation to the dark border of the corpuscle, Dr. Woodward said:

When you look at a blood corpuscle, for instance, in a microscope of high power, there appears to be a certain thickness at the edge; there is a certain dark shadow at its edge, and that has a certain degree of thickness. Now, should you measure all the shadow as blood corpuscle, or none as blood corpuscle, or shall you measure one-half of that shadow as blood corpuscle? There is always more or less uncertainty about that. I myself

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have always thought, when the objective of the highest definition was used; when we had exquisite defining power, and when, in consequence, the shadow which accompanies the corpuscle is more nearly round, you may safely bisect it with the cobweb, setting one on one shadow and one on the other, so as to take in a half shadow on each side as part of the corpuscle, but if you have taken in the whole you would get slightly larger measurements.

On cross-examination by Mr. Waller, in regard to the shadow or dark border of the corpuscle, Dr. Woodward said:

A. My opinion is that about one-half of that line is shadow.

Q. I ask you now, in your measurement, how much of that doubtful quantity that we call shadow, do you include in your measurement of the corpuscle?

A. I always include half of the surrounding dark area.

In treating blood stains with solvents of fibrin, to liberate the corpuscles for measurement, it has been found in many cases that the corpuscles were smaller than in fresh blood, unless maceration was long continued, old stains requiring several weeks before the corpuscles were in a condition to be measured. When a bi-concave blood corpuscle is placed in water, the coloring matter (hemaglobin) is dissolved out, the corpuscle swells up, thickens at the edges, becomes transparent and spherical. In this condition the diameter of the corpuscle becomes less than normal. It thus happens in examining blood stains that all corpuscles which have lost their color or have become spherical are by some experts rejected and not measured. Some corpuscles in fresh blood have much more coloring matter than others, and these corpuscles retain their color and form (as we think) much longer than paler corpuscles. We are not aware that any fluid used, or likely to be used, for softening blood stains, will cause the corpuscles to become larger than normal. This point, I admit, requires further investigation.

If the corpuscles obtained from a stain do not recover their normal dimensions, it is almost absolutely certain that their average measurement will be less than normal and *never* greater than normal. Thus, in the language of Professor Wormley, we may confidently say: "*Thus then, whilst the blood of man might, on account of contraction in diameter of*

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the blood corpuscles, be confounded with that of some animal having smaller corpuscles, the reverse could never occur."

Professor E. S. Wood in *Witthaus & Becker's Medical Jurisprudence*, Vol. II., p. 42: "There is no reagent that we know of at the present time, and no influence outside of the living body, which tends to increase the diameter of the red corpuscles."

From this discussion we claim that it has been proved beyond any reasonable question:

1. That in favorable cases blood stains can be so treated that reliable measurements and credible diagnosis of their origin can be given, as shown in the tables given and in others which might be referred to.

2. That if error occurs on account of imperfect restoration of the form and diameter of the corpuscles obtained from a stain proved [by (*a*) the Guaiacum test, (*b*) the spectroscope, (*c*) by the production of hemin crystals] to be blood, the error, if any, will be to make human blood appear like that of one of the inferior animals, and never to mistake the blood of the ox, pig, horse, sheep or goat for human blood.

3. In general, when a stain has been proved to be blood by the above tests, it may be decided certainly whether it is or is not mammalian blood. So, also, a stain from the blood of the ox, pig, horse, sheep and goat may be distinguished from human blood: thus confirming the claim of an accused person in many cases that his clothes are *not* stained with human blood. This negative testimony is certainly quite as important in many cases as testimony inculpating a prisoner.

Lastly, the expert can say, when the average of a suitable number of corpuscles from a blood stain corresponds with the average of fresh human corpuscles, that the stain is certainly not from the blood of the ox, pig, sheep or goat; and in other cases he can say, with great certainty, that a given stain is not human blood.

Such testimony, by a skilled microscopist, is of untold importance in saving the lives of the innocent, and often in

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overthrowing the plea of those who are guilty. Such testimony is quite as reliable and important to the welfare of society as that of the chemist who testifies to the presence or absence of poison that might have some resemblance to the many recently discovered ptomaines.

NOTE.—All the photoengravings in the following pages which are represented as magnified × 2560 are from negatives on wet plates by J. B. Treadwell, M. D., of Boston. Those magnified × 10850 diameters are enlargements made by the writer from the negatives × 2560 of J. B. Treadwell. Plate × 840 and all marked × 640 are from photographs made by the author, and show very clearly the appearance seen in ordinary good microscopes of moderate power at the present day. M. C. W.

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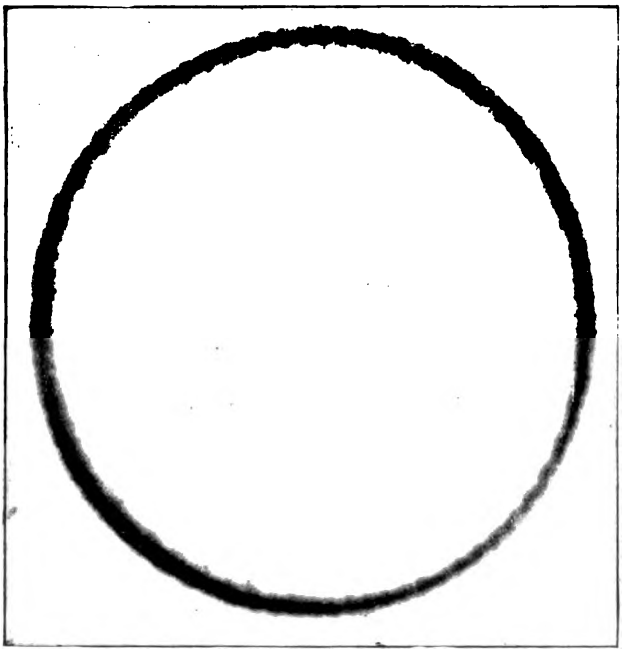
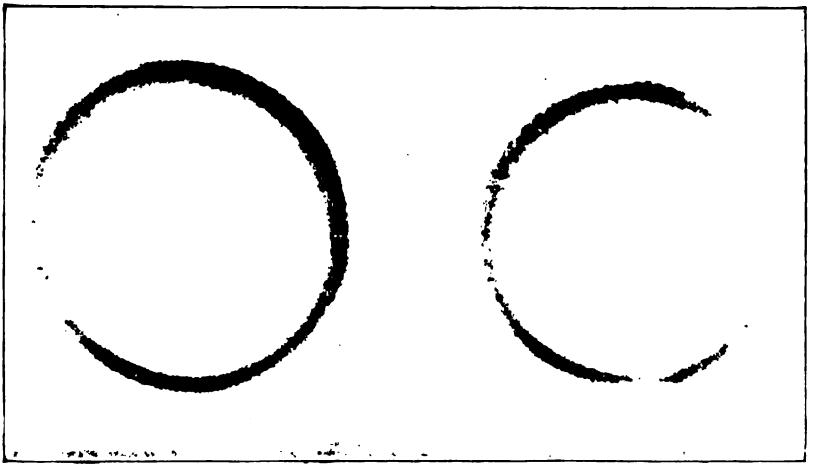


Human Blood, Red Corpuscle—X 10850.



Red Blood Corpuscles of Goat—X 10850.

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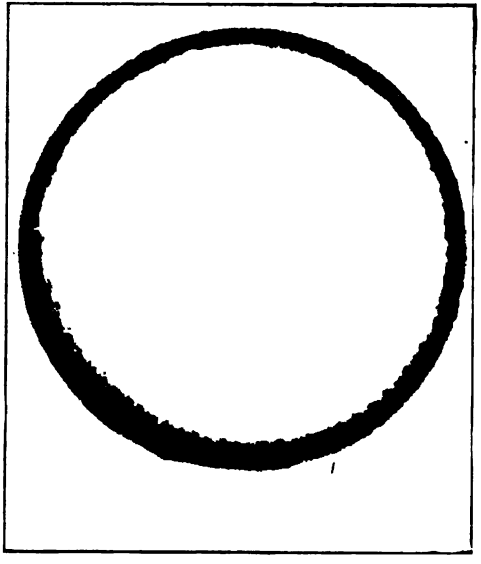
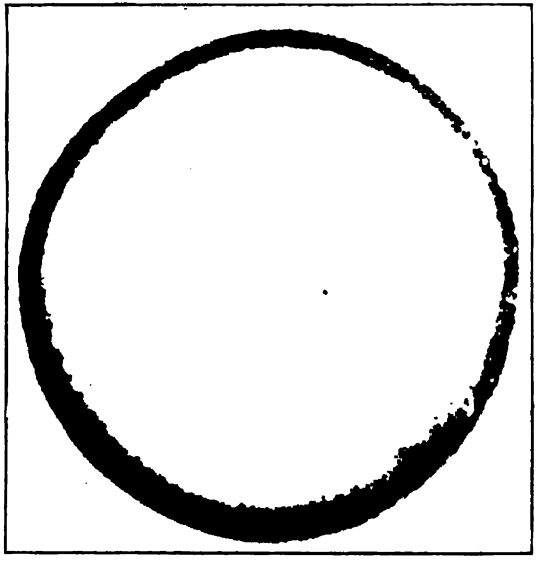


Red Blood Corpuscle, Pig—X 10850.
Small size.



Red Blood Corpuscle, Pig, Medium—X 10850.

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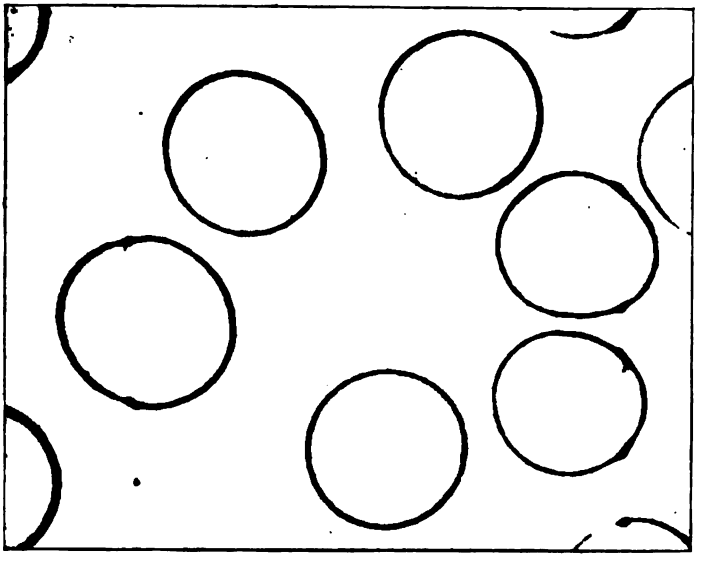
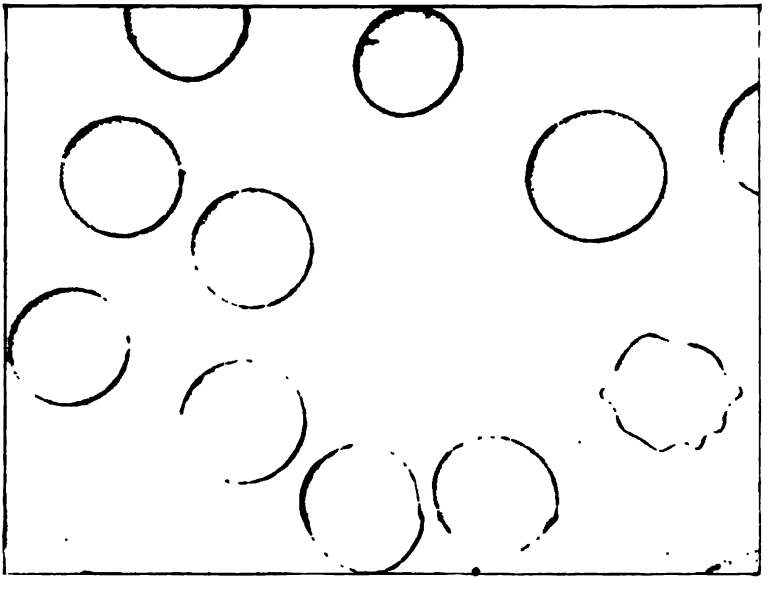


Human Red Blood Corpuscles—X 2560, with Micrometer Lines $\frac{1}{1000}$ inch.



Red Blood Corpuscles of Pig—X 2560, with Micrometer Lines $\frac{1}{1000}$ inch

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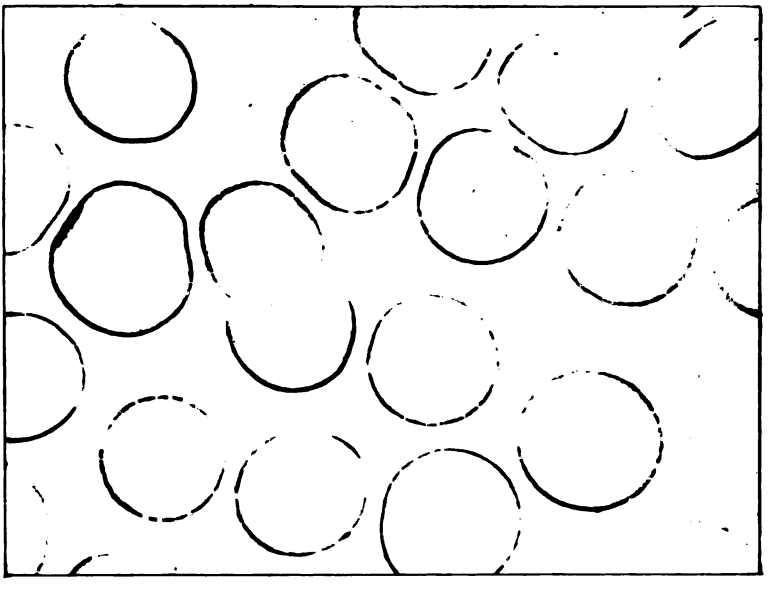
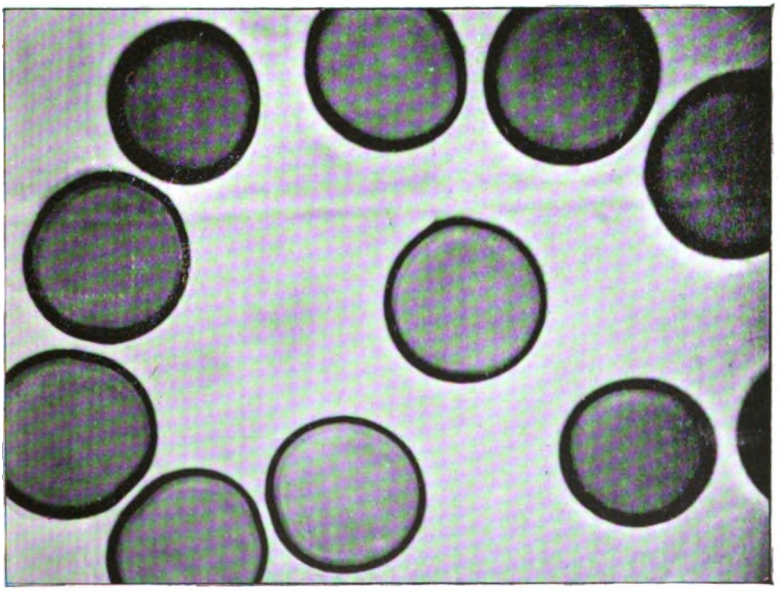


Red Blood Corpuscles of Mongrel Dog—X 2560.



Red Blood Corpuscles of Man—X 2560. As seen when out of focus.

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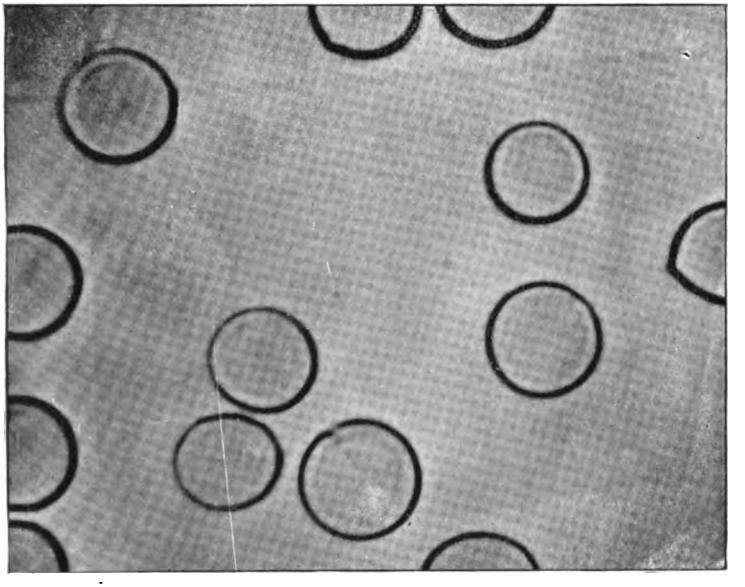
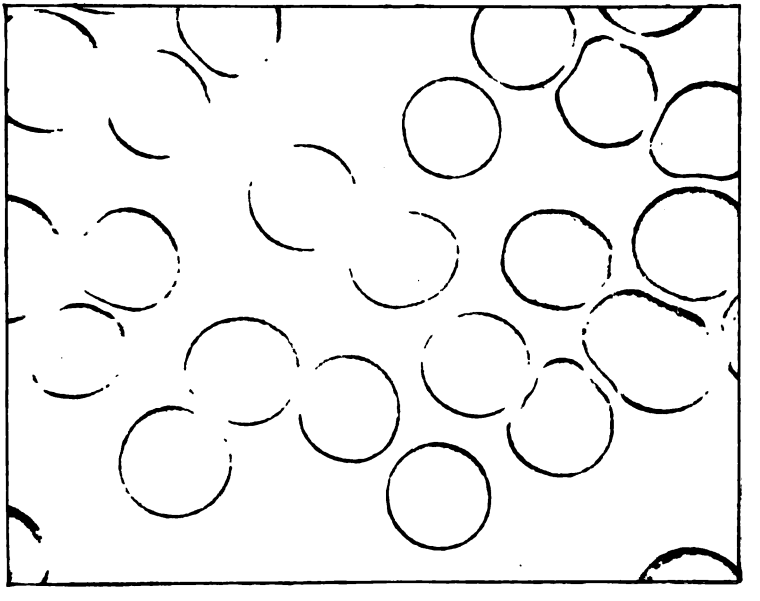


Red Blood Corpuscles of Ox—X 2560.



Red Blood Corpuscles of Horse—X 2560.

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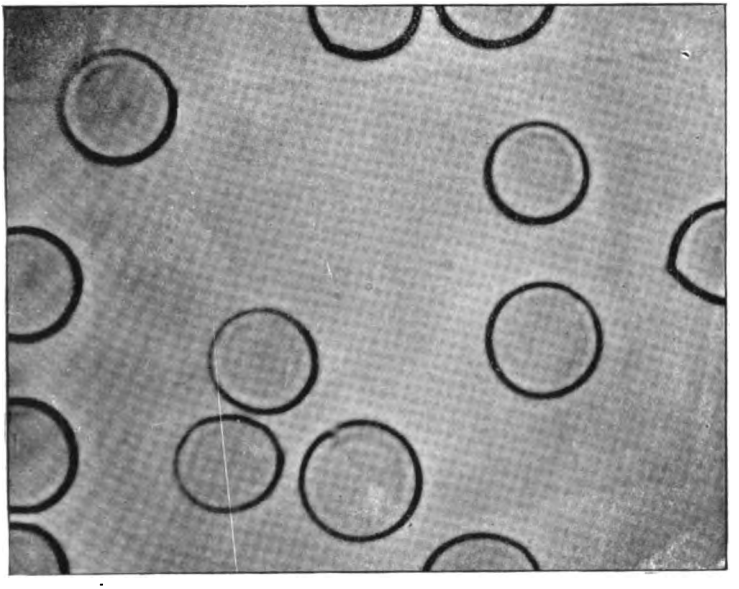
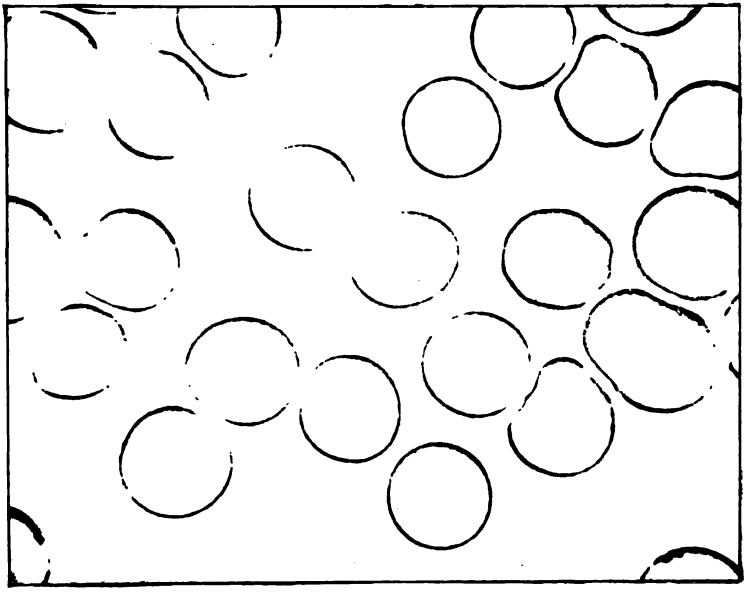


Red Blood Corpuscles of Ox—X 2560.



Red Blood Corpuscles of Horse—X 2560.

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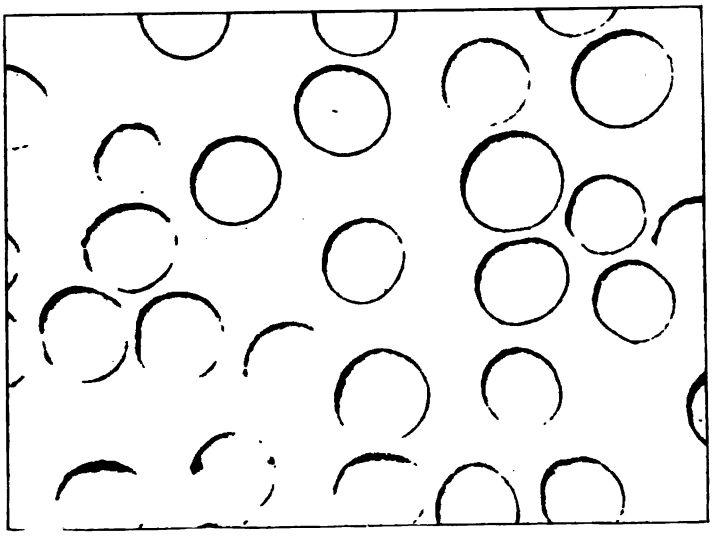
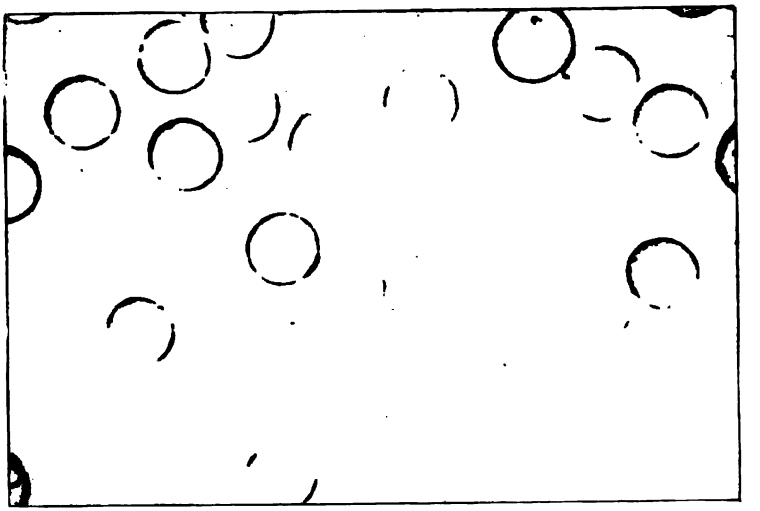


Red Blood Corpuscles of Sheep—X 2560.



Red Blood Corpuscles of Goat—X 2560.

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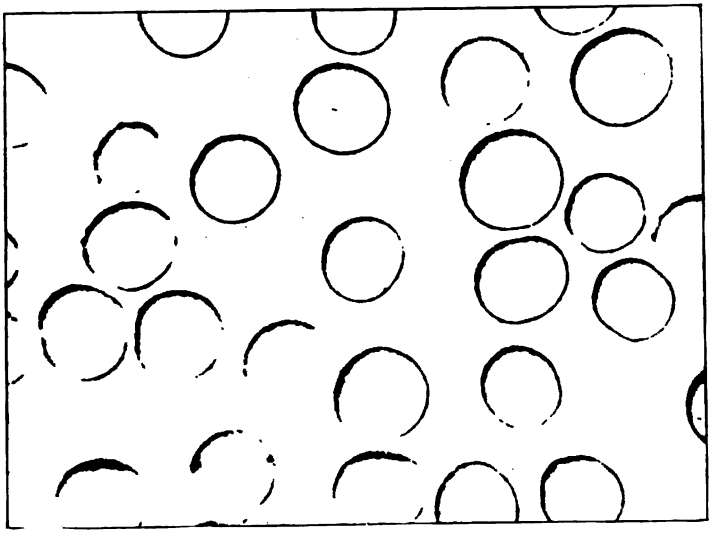
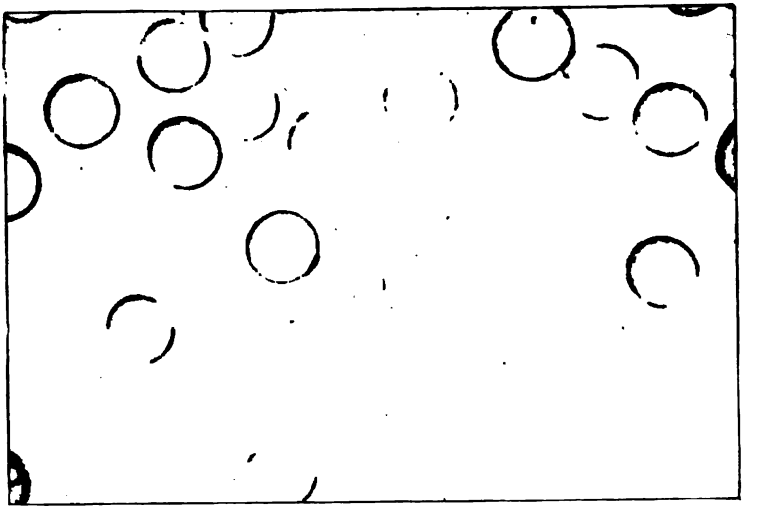


Red Blood Corpuscles of Sheep—X 2560.



Red Blood Corpuscles of Goat—X 2560.

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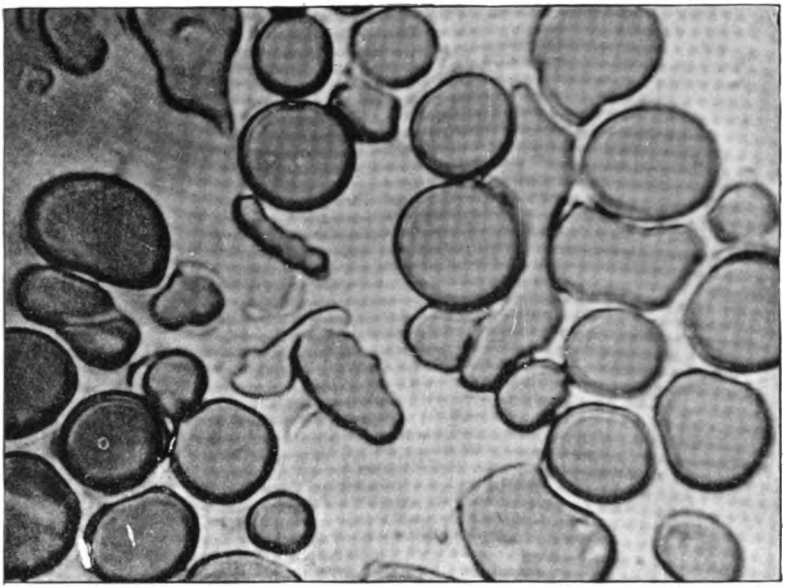
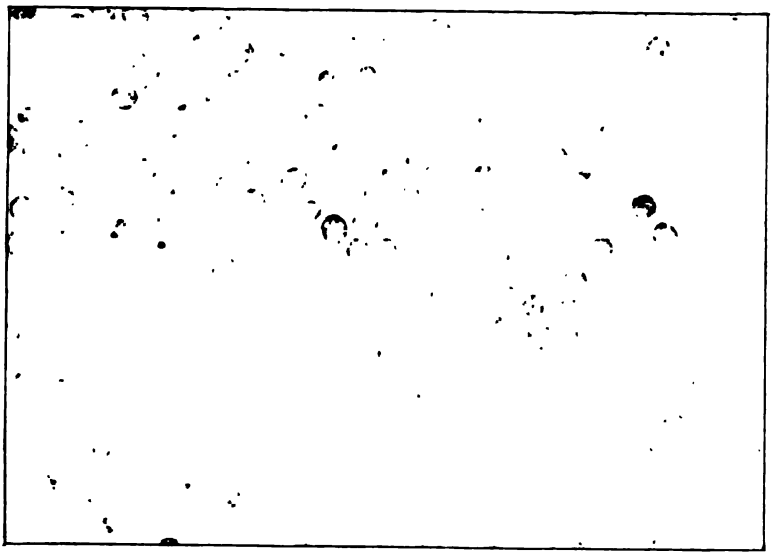


Human Blood Corpuscles (Pernicious Anemia)—X 2560.



Red Blood Corpuscles, Man (Anemia)—X 600. Very small and generally small size. Photo. by W. N. Sherman, M. D., of Merced, Cal.

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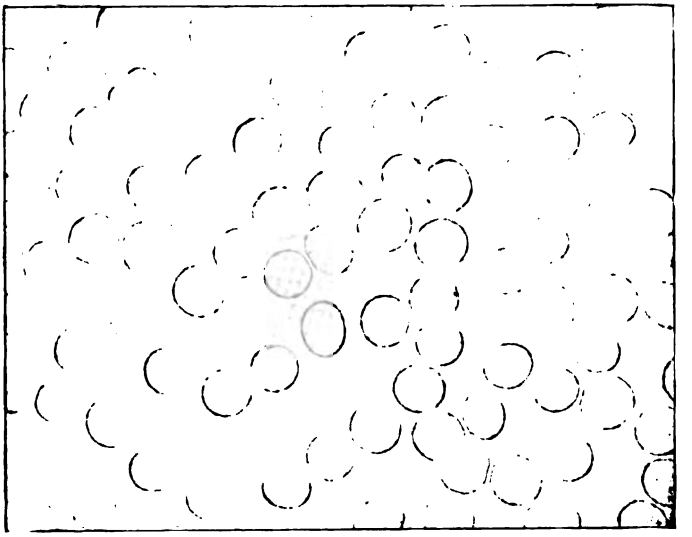
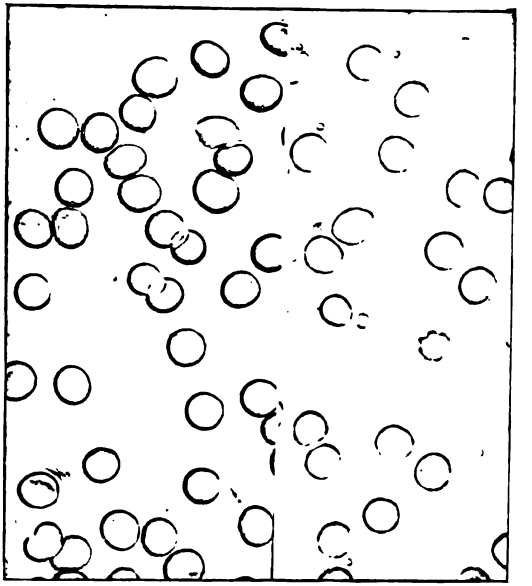


Red Blood Corpuscles (Human)—X 840.



Blood of Man—X 640. Blood of Guinea Pig—X 640.

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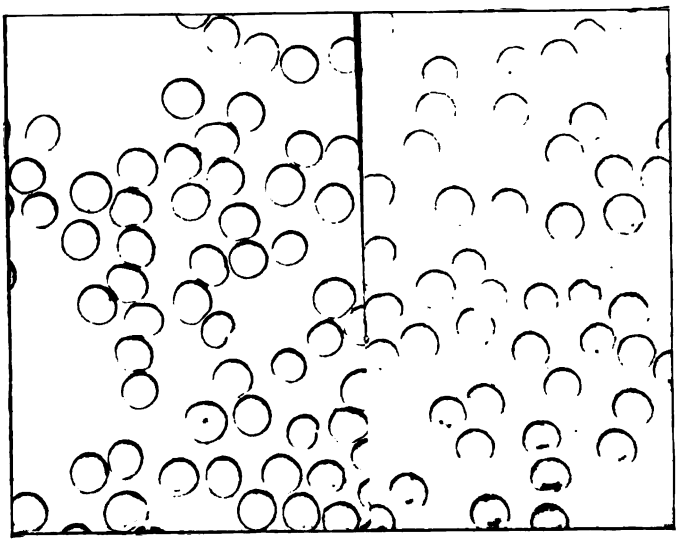
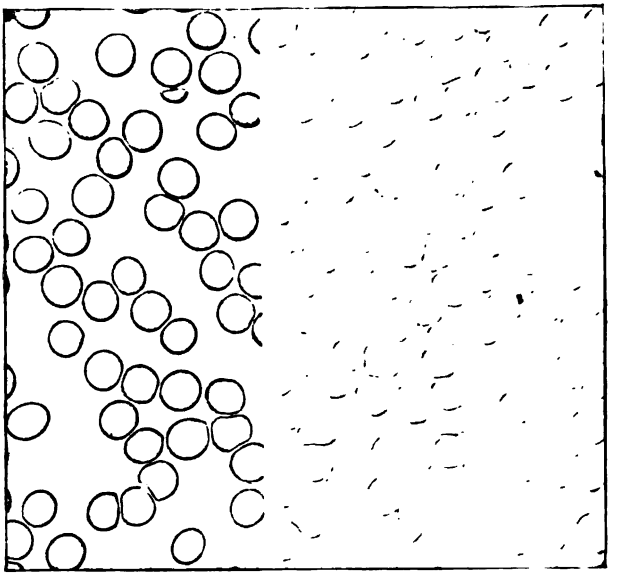


Blood of Man—X 640. Blood of Pug Dog—X 640.



Human Blood. -X 640. Blood of Bull Dog—X 640.

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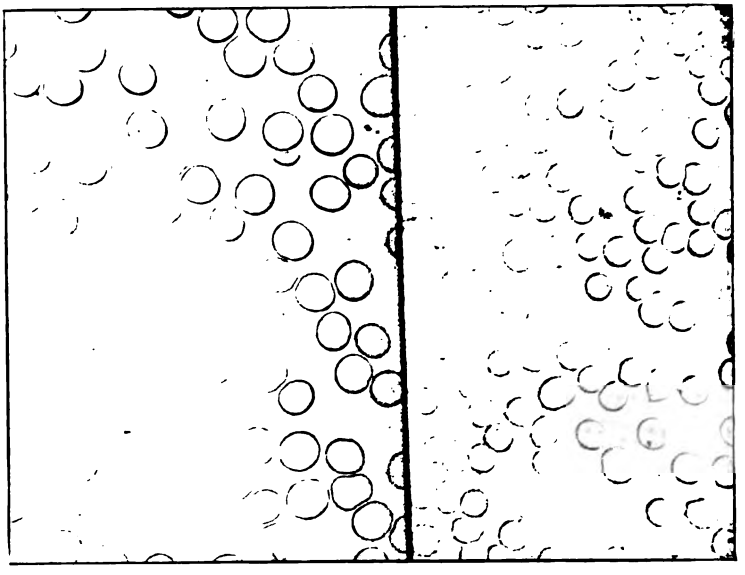
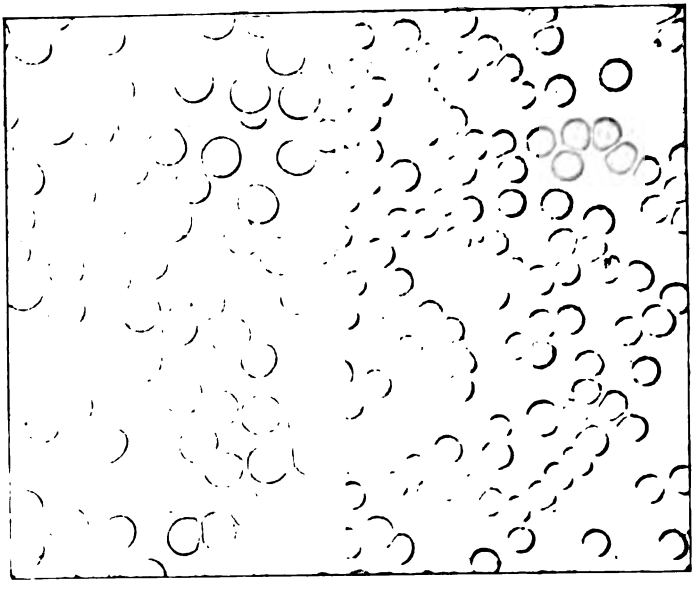


Human Blood—X 640. Blood of Young Calf—X 640.



Blood of Man—X 640. Blood of Ox—X 640.

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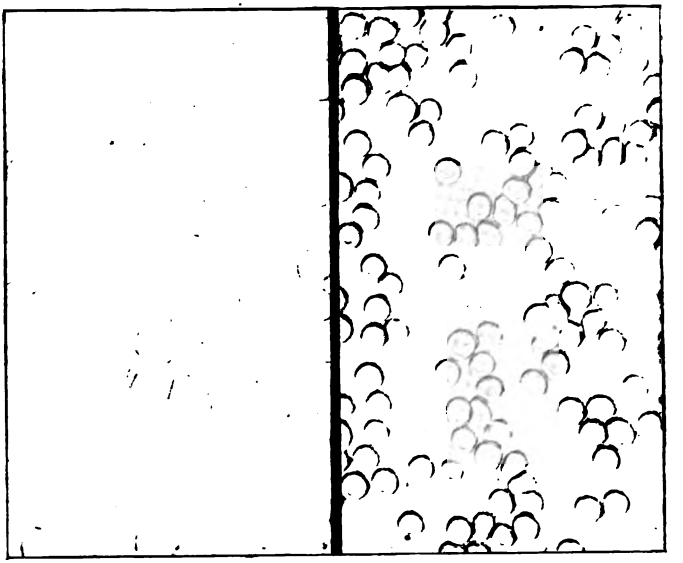
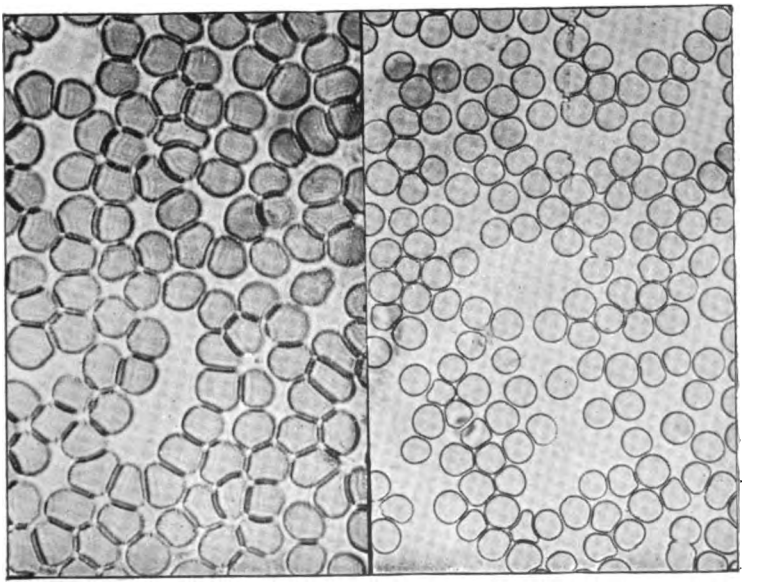


Human Blood—X 640. Blood of Cat—X 640.



Human Blood—X 640. Blood of Ass— X 640.

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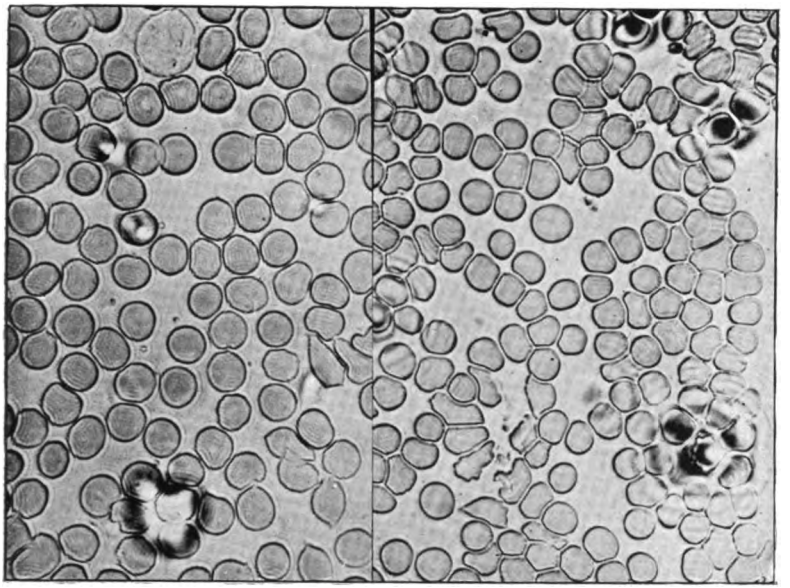
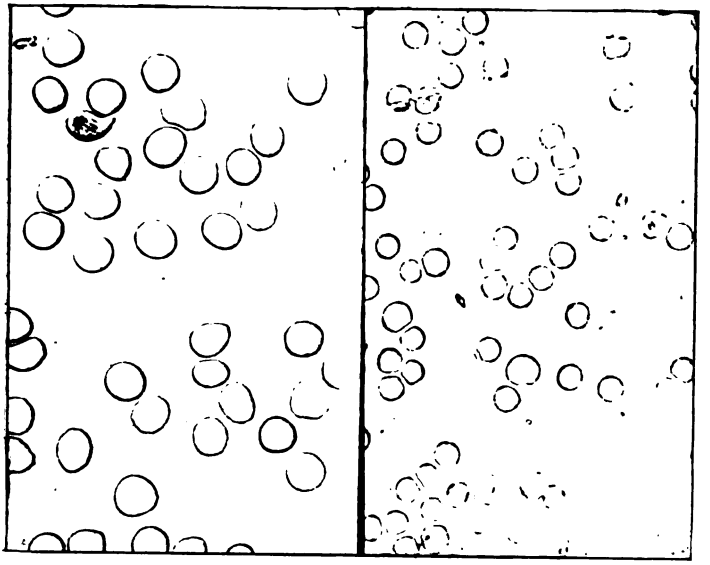


Human Blood—X 640.

Blood of Pig—X 640.



Blood of Man—X 640.

Blood of Sheep—X 640.

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YEASTS AND THEIR RELATION TO MALIGNANT TUMORS.

ALLEN ROSS DEFENDORF, A. B, M. D., Worcester, Mass.

Since the possibility of a parasitic origin of cancer, as well as other tumors, was suggested by Paget,[1] who likened the growths produced upon trees by the stings of insects to the tumors of men, almost every pathologist of any renown has investigated the subject.

A critical investigation of the voluminous literature on this subject impresses one with the fact that something is at fault, for when almost all are united in asserting the presence of "Cancer Bodies," yet few have dared to tell us just what they are. The fault lies in the fact that, with a very few exceptions, research has been conducted only on a historical basis.

Six years of investigation have shown that from the morphological standpoint, there is no hope of establishing the specific cause of these neoplasms, for, since the promulgation of Koch's laws, scientists in mycology have been loath to accept as the cause of disease, organisms which do not conform to its requirements.

The recent discovery of the fact that the class of fungi called yeasts are pathogenic has opened up a new field.

Since this discovery by Sanfelice, in 1893, the subject has aroused much interest, especially among the Italian and German pathologists, so that now the yeasts which meet the requirements of Koch's laws are to receive a large share of the attention which was so lavishly bestowed upon the supposed protozoan parasite.

Yeast fungi, also called *Blastomycetes*, are the chief representatives of the genus *Saccharomyces*. It is to be regretted that their exact botanical position has not been determined.

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Formerly they were considered as a stage in the development of filamentous fungi, but now authorities agree that they form a distinct class.

Morphologically they are round or oval cells, arranged singly, or in chains, increasing by budding or by spores, and occasionally forming mycelia. Each cell consists of a mass of granular protoplasm, surrounded by a double contoured membrane. The younger forms are, for the most part, homogeneous, while the older forms contain one or more refractive granules.

The cells as they are seen in animal tissues are somewhat different, in that they usually possess refractive granules, and a membrane varying in thickness. In some cases they are surrounded by another double contoured hyaline membrane. The protoplasm is usually hyaline. The mother cell rarely gives rise to a mycelium, which is considered an abortive form.

Much discussion has arisen in regard to these refractive granules, whether or not they are or represent nuclei. Raum says that he could never establish a nucleus. Sanfelice says that the granules cannot be compared to such a nucleus as we find in a fully organised cell. Though they stain more deeply than the protoplasm, they cannot on account of their lack of consistency be called nuclei, and besides, they have never been seen to take part in the process of budding (excepting Sirleo and Maffucci). They are persistently present in all kinds of *Blastomycetes*, but they present no constant attributes as to form, arrangement, number or changes. They were observed by Schwarm and de Legues, also by Rees and Hausen, and were called endospores, a view which Raum, Möller and Brefeld justly opposed.

Yeasts stain equally well with any of the aniline dyes, also with carbofuchsin, and hematoxylin (Büsse). The methods of staining are as follows :

The tissue is stained in toto with lithium-carmine for twenty-four hours, twenty-four hours in alcohol acidified with $\frac{1}{2}$ per cent of hydrochloric acid, treat with absolute alcohol as long as stain is removed, clear with xylol, imbed

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in paraffine, free sections of paraffine with xylol, treat with absolute alcohol, let stand from five to fifteen minutes in a flask filled with Ehrlich's solution, wash with water, treat with a few drops of 5 per cent. oxalic acid, which acts as a mordant, wash off the acid with water, treat with absolute alcohol as long as the color is removed, clear in xylol, mount in balsam.

By this method, which was selected by Sanfelice because of the beautiful colors, the tissue ground work is stained red and the parasite violet.

In Ehrlich's fluid, methylene-blue or malachite-green can be substituted for gentian-violet, when the parasites are colored respectively blue and green. If the tissue is stained in sections, the following is a good method.

The section freed of paraffine is stained ten to fifteen minutes, or one to three hours in the following solution :

Saturated watery solution of Safranine	1 p.
Saturated alcoholic solution of Malachite-green	½ p.
Distilled water	½ p.

Wash in distilled water, treat five minutes with 5 per cent. oxalic acid, wash, treat with absolute alcohol, clear in xylol and mount in balsam.

By this method the parasite is stained green or bluish-green according to the length of time the tissue is allowed to stand, the protoplasm flesh-red and the nucleus safrin-red.

In place of the staining fluid, mentioned in the above method, the following solution may be substituted :

Saturated watery solution of Safranine	5-6 p.
Saturated alcoholic solution of Gentian-violet	1 p.
Distilled water	¾ p.

Leave the specimen in the staining fluid three or four minutes, which stains the parasite violet and the tumor the high reds of safranine.

Good results can be obtained from a mixture of borax carmine and indigo carmine. After staining, the sections are treated with a saturated solution of oxalic acid. The parasites stain blue and the tumor red.

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Gram's method may be used, but the solution of iodine must be very weak, so as not to decolorise too strongly.

Yeasts are widely distributed throughout nature. Brefeld[2] found them in air, dust, plants, leaves, fruits and manure. The most difficult part in the study of these fungi has been to get them to grow on nutrient media and to isolate them, so as to procure pure cultures. Their behavior in culture growth resembles very much that of bacteria, which exhibit a few characteristics in common, but present individually their peculiar reactions in different media. They prefer acid media, in which they grow very slowly. They do not liquefy gelatine. Potato is a favorite medium, upon which their growth is usually luxuriant. A common characteristic which most of them possess is the ability to produce alcohol in sugar cultures. An idea of the manner of their growth on the different media can be obtained in the study of some of the pathogenic varieties. They do not produce toxins, but those which produce pathological effects do so by means of general infection.

Since the discovery of the yeasts, in the early part of the century, they have been identified with the process of fermentation, in which they are the active agent. It was not known until the studies of Newmayer[3] and Raum[4] that they possessed pathogenic qualities.

Raum, who inoculated ten rabbits seventeen times with the *Cereveus* and the *Ellipsoideus*, found that death resulted when large quantities were inoculated. Quantities of the parasites were found post-mortem in the lungs, where they had produced thrombosis. In one instance, a tumor was produced in the ear of a rabbit injected intravenously, which proved a caseous mass containing fungi.

Sanfelice,[5] although he had done much work on the morphology of *Blastomycetes*, as he prefers to call them, did not study their pathological effects until the latter part of 1894, when he found that a certain variety which he had isolated was pathogenic in pure cultures for guinea-pigs, sheep, rabbits, rats, sluts and pigeons.

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Because of the similarity of the colonies of this *Blastomycete* on solid media to those described by Cuboni and Pizzigonni as characteristic for the *Saccharomycetes ellipsoideus*, Sanfelice placed them in the genus of *Saccharomyces* and on account of the pathological changes he called them *Saccharomycetes neoformans.*

The culture growths were as follows: On gelatine plates the surface colonies, about the size of a pin-head and cup-shaped, were white in color, while the colonies below the surface were smaller, spherical, with a yellowish-white color and a well-defined margin. The medium was not liquefied, and whether neutral or acidified with 1 per cent. tartaric acid, offered an equally good soil. Under a microscope of high power, the cells were easily distinguished, packed very closely together. Colonies on agar plates presented the same appearances as above. In gelatine stab cultures the growth was luxuriant, both on the surface and along the stab. On the surface of slant agar, neutral or slightly acidified, they produced a finely granular, dry, non-glistening membrane, which very slowly covered the whole surface. On the potato it had the appearance of a white, lusterless membrane, with warty prominences, but a smooth border. The growth was luxuriant in milk, but produced no change in the medium. In fluid media, to which different kinds of sugars had been added—glucose, maltose, dextrose, lactose and saccharose—there was a rich growth, producing no turbidity, but oftentimes there was found on the surface a more or less thick white membrane. Dried preparations of media, stained with the watery or alcoholic solutions of aniline dyes, showed the greater number of cells to be stained intensely, throughout their entire structure. A few were stained more intensely at the periphery, while one large or several small deeply stained granules lay in the center. Examined in the hanging drop, these cultures by careful observation showed the process of budding.

The observations of Sanfelice upon the pathogenic qualities of this variety of yeast were made from inoculations of

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guinea-pigs with pure cultures. The fungi were scraped from the surface of an agar or potato culture, mixed with a small quantity of sterilised water and immediately inoculated. Cultures from each of the solid media were equally virulent. Age had no effect upon the virulence, for cultures five months old were no more virulent than fresh cultures. In all, forty guinea-pigs were inoculated subcutaneously, into the testicles, into the liver and into the peritoneal cavity, with fatal results. Those which ingested the pure culture showed no apparent effect. With the exception of the last, all died in about thirty days, only two living longer; one forty-three days, and another seventy-five days. Of those inoculated subcutaneously, there appeared in the adjacent connective tissue a tumor, which was as large as a hazel nut. Within a short time after the inoculation, the lymphatic glands in the inguinal and axillary regions began to enlarge. At the autopsy the tumor seemed to be composed of a firm white tissue and was adherent to the skin. The lymphatic glands of the axillary and inguinal regions were greatly enlarged and exhibited a tissue similar to that of the tumor. Upon opening the abdomen the spleen was found to be enlarged and covered with white spots. The liver and lungs were enlarged and with the kidney, showed similar white spots on their surfaces.

Guinea-pigs inoculated in the testicles showed at the autopsy swellings of that organ, together with changes in the abdominal cavity, much the same as those of the animals inoculated subcutaneously. In the guinea-pigs inoculated in the peritoneal cavity, the organs of the abdomen and thorax were more profoundly involved than in those inoculated subcutaneously or in defined organs. In the cavity there was a severe peritonitis, which might have been called neoplastic, and the surface was covered with a milk-white fluid. Tumors the size of a hemp seed, white in color, were found on the parietal peritoneum and the omentum. The lymph glands were enlarged.

The tissues of the guinea-pigs, when fixed, hardened,

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stained and mounted, showed interesting changes. The tumors at the site of the subcutaneous inoculation presented a nest of young connective tissue, in the meshes of which a varying number of cellular elements, which were very similar to lymph corpuscles, and whose nuclei laid mostly at the periphery of the cell body. Among these elements there were so many parasites that the tumor seemed to be due rather to the presence of the parasites than to the tissue elements.

The fungi were mostly free, and well stained, though a few were without a trace of stain. Their size varied greatly. Among the very young forms no membrane was seen; in forms of medium size there was a hyaline membrane, and in the oldest forms there was a deeply stained membrane. The last forms correspond in the fresh preparations to the forms with strongly refractive membrane.

The granules were usually deeply stained, though in some cells which had undergone degeneration nothing within the membrane had taken on stain. The protoplasm of some cells showed very clearly the two kinds of protoplasm stained to a different degree. Among the very large forms were seen some with a very clear hyaline membrane outside, but no trace of an inner membrane.

The tumors of the guinea-pigs inoculated in the liver seemed due rather to the presence of large numbers of the parasites than to any great change in tissue elements. Aggregations of yeasts corresponded to the white spots and granules scattered on the surface of the organ. These groups were found not only under the capsule but also in the parenchyma.

The tumor of the great omentum in the guinea-pigs inoculated in the peritoneal cavity presented the same structure as those noted above—namely, numerous fungi lying between the meshes of a loose connective tissue, which was in some places thickened, but otherwise normal. Many parasites were found also in the pancreas and salivary glands.

In the kidneys they were more numerous in guinea-pigs

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inoculated into the peritoneal cavity than into the subcutaneous tissue or into special organs. The situation by preference seemed to be in the cortex and inside of the increased connective tissue, corresponding to the white spots seen by the naked eye. Groups of the parasites had destroyed parts of the convoluted tubules. They were seen in the loops of the glomeruli, and, as a consequence of the rupture of the glomeruli, had escaped into Bowman's capsule. By this it was proved that in the kidney, at least, they spread through the blood.

The number of parasites was much larger in the lungs of the guinea-pigs inoculated in the peritoneal cavity than subcutaneously. They were found in larger numbers in the lungs than in any other organ. They laid in the spaces between the alveoli, and caused a considerable increase of tissue.

This variety of yeasts was of less importance on account of the pathological conditions which it produced than because of the morphological similarity which its forms presented to the structures, which have been described by the many authors on malignant tumors of men as belonging to the Protozoa.

Sanfelice has since discovered several varieties of yeasts, which he has not yet described fully, but which he claims belong to the group of *Neoformans*. Whether or not they are pathological he has not determined. He has, however, succeeded in two cases, one of an epithelioma of the lip and the other in an epithelioma of the mammary glands, in securing pure cultures of yeast fungi, which he has proved by inoculations to be pathogenic to animals.

Sanfelice[6] remarks upon the neoplastic growth in a slut inoculated in the mammary glands with a pure culture of another yeast, that a tumor was produced at the site of inoculation, and also that there were metastatic tumors in the intestines and in the spleen. In the connective tissue which surrounded the gland, groups of cells were noticed, some of which possessed a large nucleus pressed to the peri-

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phery, and other smaller nuclei similarly disposed. The cells were packed together in rows which crossed each other, giving the appearance of a fibrous carcinoma. From these groups radiated outward rows of cells which seemed to burrow between the connective tissue fibers. There was no evidence of inflammation. The parasites could not be found in the center of these groups, even by the most careful searching. Cells at the periphery, however, contained them, where they appeared to be in the early stages of growth, mostly without membranes. The histological structure of the new growths in the other organs was of a similar character to that in the breast. The yeasts were situated in the cytoplasm of the cells, were round, and possessed the characteristic double contoured membrane. With this variety an inoculation was made between the leaves of the comb of a rooster, and caused a neoplasm, which approached in character a sarcoma. The sections of the tumor showed that the larger number of the yeasts was in the central part of the tumor. Many of the parasites were degenerated, and so stained lightly. The tissue of which the tumor was composed consisted of young connective tissue elements, among whose bundles lay many cells with intensely stained nuclei and sharply defined cell bodies. In some places these cells produced thick bands, among which could be perceived isolated yeast cells.

In May, 1895,[1] Sanfelice isolated a variety of yeasts from an ox which had died of primary cancer of the liver and secondary invasion of that process into the whole lymphatic system. Pieces of the enlarged glands were applied to the different fluid and solid media and a culture established. By teasing pieces of the glands in a fluid consisting of equal parts of glycerine and water, yeasts were found in large numbers, of different sizes, mostly round and possessing a refractive membrane. Most of them contained refractive granules. Near these parasites were found many others of an appearance which the *Blastomycetes* ordinarily give inside of the cells. They were homogeneous and of a glassy refrac-

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tibility, or of that sort of refractibility which calcareous bodies present in pathological tissues. Besides these forms there were irregularly formed masses with a sharp outline and a calcareous refractibility, which he at first considered to be calcareous degeneration products.

Plate cultures of gelatine showed colonies of yeasts which on the surface were round, white and of the size of a pin-head, but beneath were smaller, spherical, sharply defined and of a yellowish color. Stab cultures in gelatine showed colonies both on the surface and along the stab. On slant media the colonies were white, elevated and of a dried appearance. Examined microscopically, they appeared as round cells, of different sizes, according to the age of the culture, and with glistening granules ; but the glassy-looking bodies, described above, could not be found,

From the culture growths and the microscopical appearance, the yeast seemed in no way different from the pathogenic variety, named *Saccharomycetes neoformans*, and described above.

Guinea-pigs inoculated subcutaneously with an emulsion of pieces of the glands and sterilised bouillon died in two months and showed the following pathological conditions :

The axillary and inguinal lymph glands were enlarged ; the great omentum showed a few small nodules the size of a hemp seed ; a few were seen also in the spleen ; the lymph glands of the abdomen were also enlarged ; small nodules were seen in the lungs ; the liver, intestines and brain exhibited no changes. When inoculated intraperitoneally, conditions identical to those just described were found.

Small pieces of the lymph glands of the dead guinea-pigs, teased and examined fresh under the microscope, showed parasites both free and enclosed in the cell elements of the gland. They disclosed the characteristic membrane, while some of them had a more or less hyaline halo surrounding the membrane. The protoplasm was homogeneous with the usual number of refractive granules. Besides these normal forms were seen others, which refracted the light like

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glass. These were degenerate forms of the fungi. In the kidney they were present in such numbers that upon section it seemed as if the organ was full of calcareous deposits. It was noticed with those guinea-pigs which died a greater number of days than usual after the inoculation that the reaction on the part of the tissue was greater, from which fact it was concluded that after some time, if the animal survives, the parasites tend to degenerate and disappear.

Sanfelice gave this variety of yeast fungi the name of *Saccharomycetes litogenes*, from the appearance of calcareous degeneration which it gave. The *Saccharomycetes litogenes* were pathogenic also for white rats, rabbits, sheep and cattle.

Shortly after the first contribution of Sanfelice appeared, Maffuci and Sirleo[8] described a pathogenic yeast fungus which they obtained from a guinea-pig that had died of marasmus. They were successful in establishing pure cultures, which upon inoculation into guinea-pigs gave fatal results.

The parasites were found in the lung, kidney and lymph glands. They observed that the structure of the cellular tissue was the same in all of the above organs, and similar to that which was described above, in Sanfelice's investigation. They noticed, however, a greater activity in the formation of new structure in the lungs than in the other organs. This dissimilarity may be explained by a longer time, which the parasites had acted before the death of the animal, which fact was not mentioned.

Otto Büsse[9] discovered a pathogenic fungus in the study of a malignant tumor of the tibia of a woman, which during life was diagnosed as a sarcoma, but by post-mortem examination appeared as a form of chronic pyemia, with a number of giant cells. They appeared in the pus among the giant cells as small, clear, refractive, round or oval bodies, of a size varying between that of a small nucleus and a liver cell. They were also found as cell enclosures within the giant cells. At first, they were looked upon by Professor Grawitz as belonging to the group of Microsporidia, being

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Cornalia's bodies or *Coccidia*. Later, as the result of positive culture, they were identified by Professor Löffler as a kind of pathogenic yeast fungus. The possibility that he might be dealing with a variety of *Coccidia*, as the descriptions and drawings of Darier and Wickham suggested to him, led him to try a culture in sugar bouillon, which produced alcohol. This left no doubt that the parasites were yeast fungi.

He injected into the marrow of the tibia of a rabbit a small piece of the tissue taken from the tumor. The leg became gangrenous in three days and was amputated, when it was found that the whole length of the tibia was beset with yeast cells, which proved that the yeast cells had the ability to multiply in human tissues. Some of the pus from the tumor was injected into the peritoneal cavity of a rabbit, with fatal results in three days. The mesenteric glands were much enlarged and in them were found the parasites in large numbers.

The fact that Büsse published his results a month after his first inoculation, before some of his test animals had begun to show the effects of the inoculation, or before their tissues had been examined histologically, prevented him from drawing any other conclusion than that the yeasts were capable of multiplying in animal tissues.

Ajevoli[10] and Pianese[11] have done work which could not be obtained.

Corselli and Frisco[12] succeeded in isolating a pathogenic yeast from a case of sarcoma of the mesenteric glands with a milky exudate. At the autopsy a neoplastic mass was found, consisting of enlarged mesenteric glands with many small tumors, of the size of a lentil, situated on the diaphragm and small intestines, and a quantity of milky fluid in the abdomen and thorax, similar to that seen during life. Microscopical examination of the fluid, made during life, showed cellular forms of different sizes, some small, others the size of a liver cell, mostly round, which were isolated or in groups, very often of four. Most of them showed one or more granules. They stained by the usual methods.

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Inoculations of the pathological material were made upon the ordinary media, but neutral or alkaline focus was the only one upon which a culture grew. From this medium colonies of the *Blastomycetes* were isolated and transferred to gelatine, simple agar, glycerine agar, and sugar agar, and also bouillon, where the culture flourished. A microscopic examination of the pure cultures showed the fungus to be of a larger size than those in the pathological material, though in respect to the refractive granules no difference was noticed. The cells were surrounded with a thin membrane. The protoplasm was usually homogeneous, with a granular appearance at some places. In the hanging drop were seen small granules, which increased in size and approached the appearance of round bodies, and which later became still more prominent on account of their different refractive contour and their different disposition toward stains. Finally, they burst the membrane and became free, assuming the first stage of the parasite. The more usual means of propagation was that of budding. Another unusual form was that of forming a rod-like appendix, which showed the tendency for producing a mycelium.

The results of the inoculation of guinea-pigs, rabbits and dogs, with both the pathological material and the pure cultures, were uniformly fatal. The guinea-pigs inoculated with five cubic centimeters of the milky fluid, or two cubic centimeters of a ten-day bouillon culture, died in about thirty days. The lymphatic glands of the mesentery were greatly enlarged. At other places in the mesentery appeared numberless groups of ball-like nodules. Similarly many little nodules were found along the line of the lymph channels, in the axillary and inguinal regions. A microscopical examination of these nodules disclosed a structure similar to that found in the woman, except that mycelial forms of the fungi were very rare. Rabbits inoculated with five cubic centimeters of the fluid, or five cubic centimeters of the bouillon, died in from thirty to thirty-five days. The same appearance, macroscopically and microscopically, were noted in the guinea-pigs.

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Of the dogs which were inoculated intravenously and intraperitoneally, one died in five days without any apparent changes. The parasite was regained only from the blood of the heart. One of the dogs inoculated intraperitoneally, showed a tumor of the mesentery, the size of a small hen's egg, and lymph nodules as large as white beans along the spine and in the peribronchial glands.

The facts that negative results were obtained in the cultures of the parasites in the juices from fruits, that there was a slight tendency to grow on acid media, and a failure to produce alcohol in saccharin fluids, influenced the investigators to conclude that during their life as parasites in the tumors of animals, they naturally suffered a change in their mode of life, and acquired a character qualified for their parasitic existence in animals.

From these observations it follows that this *Blastomycete* caused among the inoculated animals neoplastic growths of a malignant character.

Observations have been made by Roncali[13] upon *Blastomycetes*, but only from a histological standpoint. He found them in the cytoplasm of certain cells, similar to those of a *corpus luteum*, which were present in the stroma of an adenocarcinoma of the ovary. The tumor, the size of a child's head, consisted of three cysts, which contained a slimy fibroserous fluid. On the inner wall of the cysts, and floating in the fluid, there were found little bodies of different sizes with a cauliflower appearance. Histologically, these neoplasms consisted of a ground-work of connective tissue, from which there were numerous ramifications lined with cylindrical epithelium. In the enlarged cell bodies of the epithelium were found the parasite. The cell protoplasm was granular and the nucleus usually apparent. In some cells the nucleus was pushed to one side and hardly distinguishable, and in others the protoplasm appeared as if vacuolated. The parasites in great numbers were both within the cells and free in the connective tissue, most of them, however, in the former condition. Very many of the cells contained a single parasite,

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though some of them had as many as ten. These cells were swollen, with the nucleus shoved to one side. The form of the parasites was round, rarely oval or kidney-shaped. They varied in size, some being larger and others smaller than the nucleus of a cell. They were always within the cell protoplasm, never within the nucleus. A capsular membrane surrounded them, which appeared as a hyaline circle. The younger forms possessed no membrane. No nucleus was found. The younger and growing forms were more numerous than the old and degenerate ones, as the latter, he considered those forms which did not stain, but appeared pale and refractive.

Twelve weeks after the operation for the removal of the adenoma, the patient died. At the autopsy many metastatic tumors of the diaphragm and omentum were found. The omentum was thickened and on its surface appeared several nodules of different sizes and of greyish-white color, which, upon being incised, appeared compact and of pearly whiteness. By microscopical examination, many parasites were found in these metastatic tumors. They were similar to those found in the primary lesion, with the exception that only a few older forms were seen. A number was extra-cellular, when they appeared in groups of from seven to thirteen, surrounded by leucocytes or epithelial elements.

Roncali failed completely in the production of pure cultures. From the histological examinations, he concluded that these cells were a variety of *Blastomycetes*, because of their close identity to forms seen by the many other investigators. Naturally, Roncali comes to the conclusion that there is an etiological connection between the primary adeno-carcinoma and the secondary metastatic tumors of the omentum and the mesentery, from the fact that the parasites which were nested between the bundles of connective tissue and in the swollen epithelial cells of the tumor, are likewise found in the mestastic tumors, both in the epithelial cells and among the fibers. Another important fact is that only younger and

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growing forms were present in the metastatic tumors, and the older and degenerate forms in the primary tumor.

Roncali,[14] in trying to establish the etiological relation of yeast fungi to sarcoma, made histological examinations of five sarcomas. The first, a large round celled sarcoma of the crest and body of the ileum; the second and third, spindle celled sarcomas somewhat melanotic, from the orbit; the fourth and fifth, large spindle celled sarcomas of the superior maxilla, also somewhat melanotic. His results were as follows: in five tumors from different locations and in different tissues, he found in every case parasites. They resembled morphologically the forms which Sanfelice had described. They took the stains of the *Blastomycetes* and withstood the action of acids and alkalies. They were found both inside and outside of the cells. They multiplied by budding. Some were found in youthful stages without a membrane, with highly colored protoplasm; and others older, with a thick membrane and a somewhat chromatic heterogenous protoplasm which had lost the faculty of staining.

Colpi,[15] claims to have found a variety of yeasts in a case of chronic endocervicitis, which acted as the etiological agent. But from the fact that he could in no way produce fermentation with it, authorities believed either that it was not a yeast, or if it was, it represented a degenerate form.

Other Italian authors, Claudio, Fermi and Aruch,[16] have worked on this subject and have concluded that there is a pathological yeast, whose chief characteristic is to produce neoplasms of a chronic nature, whose cellular elements had the property of wandering away from the neoplasm into the lymphatic glands. They isolated their yeast from a horse affected with an infectious kind of lymphangitis, and found by inoculations that it was markedly pathogenic.

Lydia Rabinowitsch,[17] under the direction of Koch, has studied the action of fifty different varieties of yeast on test animals. Of these only seven were found to be pathogenic, and none of those were from culture yeasts. Inoculations of the culture yeasts proved fatal in mice and rabbits only after

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immense doses, and even then only a few parasites were found in the tissues.

She concludes from her research that none of the pathogenic varieties were identical to those which had been described by the other authors. In her cases it is probable, from the fact that the yeasts were more plentiful in the blood than in the organs, that death was due to the infection. In the dead animals most of the cells were found free in the tissue, only a few being seen in the cell elements.

On a gelatine plate culture of pus, taken from an appendix removed by Professor Carmalt, a colony of yeast fungi was found, which the author studied. In gelatine stab culture the growth appeared in the form of the needle along the whole stab. On gelatine plates they formed a small white globular colony, which spread very slowly without liquefying the gelatine. On slant agar the colony, which spread more rapidly than on gelatine, presented a pearly white appearance. On potato the growth was quite luxuriant, giving a yellowish-white appearance. Examined in the hanging drop, the cells were found to be mostly round or oval, consisting of a granular mass of protoplasm, which was surrounded with a double contoured membrane. Stained with methylene-blue, the protoplasm of the cell showed deeply-stained granules. Because of the lack of material, no inoculations were made to ascertain whether the yeast was pathogenic. Consequently the results are of little value; however, had a like discovery been made two years ago it would have been regarded as an accidental inoculation from the air. Now, with the knowledge that yeasts have been found in the pus of a sarcomatous sub-periosteal tumor and in a sarcoma accompanied with *Ascites chylosus*, there is reason to believe that this specimen of the yeast fungi, when inoculated into test animals, may prove to be pathogenic.

Reviewing the whole field of research on this subject, what conclusions can be drawn, first, as regards the yeast fungi in their relation to animal and human tissues; and second, in regard to their relationship to malignant neoplasm?

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(*a.*) Yeast fungi are known to invade the tissues of animals and men, where they subsist in the same manner as bacteria.

(*b.*) Certain varieties of yeast fungi are pathogenic through infection to animals, among which are dogs, pigeons, horses, cats, rabbits, guinea-pigs and mice.

(*c.*) They are present in diseased conditions of the human tissue.

Before trying to establish any relationship between the yeast fungi and malignant tumors, it is necessary to review, with that idea in mind, the work of the several investigators.

The investigation of Roncali is, perhaps, of the least real value in this problem, because he dealt with the question from a histological standpoint only. His case of adeno-carcinoma of the ovary with metastatic tumors in the mesentery, presented what he believed to be yeast fungi. The fact that they are fungi he infers from their marked similarity in shape, size and appearance under the influence of stains, to the forms which Sanfelice has proven to be yeasts. His investigation of the five cases of sarcoma was of the same nature. In all cases he found bodies identical to those which other authors have isolated from sarcoma, inoculated into test animals, and caused tumors similar histologically to the original one.

Büsse succeeded in isolating a pure culture of a yeast, from a sarcomatous sub-periosteal tumor of the tibia. This he found to be pathogenic to animals, and in the case of an inoculated dog to cause a tumor. His observations were not very complete, as he published his paper before the tumor had been examined histologically. Although there is no direct evidence as to what the tumor was, yet we are warranted in inferring, from results obtained by other men, that if it had been examined the presence of yeast fungi would have been determined.

The observations of Maffucci and Sirleo are of importance, because they succeeded in producing neoplasms in all of their test animals, for which their fungi were pathogenic. They

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were among the first to suggest the possibility of the yeast fungi being the cause of malignant tumors.

Corcelli and Frisco present results of the utmost importance. They succeeded in procuring a pure culture of a *Blastomycete* from a tumor, which, both during life and at the autopsy, showed itself to be a malignant neoplasm. This caused, among the test animals, tumors which were identical to the original tumor, both in location and structure. It is certainly easy to establish an etiological relationship here. Furthermore, with the discovery of this parasite, equipped with the power to cause malignant tumors, there is opened a new field in the study of *Ascites chylosus* and the connection which exists in man between these tumors and the exudate.

To Sanfelice we are indebted, more than to any other investigator, for our present knowledge of pathological yeast fungi. The uniformity of his success in producing tumors among the test animals with all of his pure cultures is most remarkable. This fact, together with the notable similarity of the yeasts in the tissues of the animals which had been inoculated, to the so-called *Coccidia* of many authors on the cause of cancers, led him to investigate malignant growths, with the result that he succeeded in making pure cultures of yeasts from a case of carcinoma of the breast and one of carcinoma of the lip. These cultures, by inoculation, proved pathogenic.

The results of Lydia Rabinowitsch, who found only seven pathogenic varieties of yeast among fifty, which in no case produced tumors, somewhat temper the results of the other investigators. None of her yeasts produced neoplasms, and none of them were pathogenic to guinea-pigs although to other animals. These results are not of so much importance when we consider that of all the yeasts known four years ago only two varieties were found by Sanfelice to be pathogenic.

The results of Fermi and Aruch, who produced a pure culture from a horse affected with an infectious lymphangitis and proved it pathogenic, add evidence not only to the patho-

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genic character of yeast fungi but also to their ability to produce malignant growths.

Almost all the investigators of yeast fungi have alike been impressed with the marked similarity which exists between the yeasts as they appear in tissues and the numerous forms of cancer bodies, as they have been described and pictured by the many writers on the parasitic nature of malignant tumors, especially carcinoma. With this fact in view, it seems best to consider them here and, if possible, to show that they represent in the tissues a variety of yeasts.

After the suggestion offered by Paget, in 1887, at various times, several authors, as Rappin,[18] Scheurlein,[19] Brault,[20] Senger,[21] Baumgarten,[22] Rosenthal[23] and Kubasoff[24] described varieties of bacteria as the specific cause of cancer, but the discovery of similar microörganisms in non-cancerous tissue led to the belief that their presence in those cases was merely an incident.

The apparent protozoan nature of certain bodies in epithelial formations, such as *Molluscum contagiosum*, described originally by Virchow[25] and a number of other investigators, among them Rivolta,[26] Bollinger,[27] Neisser,[28] suggested the likelihood of the existence of cancerous bodies in the allied cancerous formations.

In 1888, Pfeiffer[29] described Sporozoa, and Malassez,[30] in 1889, announced the discovery of bodies resembling *Coccidia oviformes* in the cells of the epithelioma of the jaw.

About the same time Thoma[31] described small cell-like bodies found in the epithelial cells of glandular cancers of the rectum, breast and stomach, all of which, and especially the latter, might be, from their description, easily confounded with yeasts.

Albarren[32] found round bodies in the epithelia, which he described as psorospermes, a greater part of which he says had the form of round or little egg-shaped cells, also possessing a single central nucleus. Some of the parasites were clearly encapsulated with a membrane of varying thickness. There were others which were very refractive and homogeneous with-

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out nuclei. One could scarcely need a better description of the *Blastomycetes* found by Sanfelice in the tumors of the guinea-pigs which had been inoculated with a pathogenic variety of yeast fungi. The latter part of the description tallies precisely with the form which the degenerated *Blastomycetes* take on.

Darier[33] found, in a certain skin disease, *Psorospermosis follicularis vegetaris*, round, intra- and extra-cellular bodies with a thick refractive membrane. His figures correspond exactly with drawings of yeasts.

Wickham, a scholar of Darier, found similar bodies in cases of Paget's disease. His drawings also were like those of yeasts.

Nils Sjobring,[34] who had been studying the process of nuclear division in cancer cells, had noticed certain bodies which he called Protozoa. The cycle of development, so well described and pictured by him, can be easily explained by stages in the development of the yeast fungi.

These descriptions of Albarran and Sjobring were soon corroborated in publications of Balbiani,[35] Hacks,[36] Wright,[37] Strobe,[38] Steinhaus[39] and Kursteiner.[40]

Soudakewitch[41] found in the cells and cell nuclei of glandular cancers small round or oval bodies, sometimes with a membrane about them. Metchnikoff, who also examined them called them *Coccidia*. His drawings are very similar, in some respects, to those of authors on *Blastomycetes*.

Sawtschenko and Podwyssozki[42] failed to notice parasites within the cell nucleus, but in most cases found them in the cell bodies.

Sawtschenko[43] described the apparent life history of the so-called Sporozoa from sections of a cancer of the lip. Here, also, both the descriptions and drawings of these stages resembled very closely conditions of the yeasts.

Foa[44] studied especially carcinoma of the breast and mentioned small bodies, which were usually homogeneously but sometimes contained deeply-stained central granules, also larger forms, which might reach the size of a white blood

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corpuscle. They were surrounded with an intensely stained capsule. He also described forms which, had he not been looking for *Coccidia*, might have been explained as *Blastomycetes* in the process of budding. Other forms, which present a striking resemblance to these, were those which he described as not staining. Whether this was due to degeneration he was unwilling to say.

Koretneff,[45] Kurloff,[46] Smith[47] and Clarke[48] describe forms of a gregarine-like body, which they claim is the fully developed form of the parasite, and to which the name of *Rhopalocephalus carcinomatosum* was given. An undeveloped stage of this parasite, which was found most frequently in the sections, resembles very much the yeast fungi.

These are the only writers who have recognised gregarine-like forms as a factor in the etiology of the epithelioma. From the fact that they were found only in relation to pearls of epitheliomata of the lip or penis, it seems as if they had mistaken degeneration products for genuine organisms.

Buchardt[49] studied cancers of the mucous membrane and found in most of them intracellular structures surrounded with a clear capsule.

Ruffer and Walker[50] confirmed the investigation of continental workers and, later, Ruffer[51] announced that he had seen every stage in the life history of the Protozoon of cancer, from the time the parasite appeared as a spore in the nucleus until it left the latter as a young, fully formed parasite.

Galloway[52] has recently announced his belief in the protozoan nature of the various bodies described, on account of their apparently organised structure, their staining reactions and their analogy to well-known Protozoa. He figures them as small round masses of protoplasm, which may become encysted and then break up into a number of sporules.

Of all the authors who have busied themselves with the parasites of malignant tumors, Russel[53] is the only one who has described a fungus as the real cause. These, because of their affinity for acid-fuchsin, he calls "fuchsin-bodies," and states that they may be found in cancers without exception.

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These bodies may occur in groups of two, three, four or more; generally have a clear space about them; are bounded by a capsule; are spherical in shape, measuring from four to twelve mikrons in diameter, and are structureless in appearance. He judged that they grew by gemmation. They were found in the more rapidly growing part of the tumor.

Realising the weakness of a parasitic theory of the origin of cancers, which is unable to comply with Koch's laws, several investigators have attempted to inoculate cancerous tissues into test animals. Hanan made a successful inoculation of carcinoma taken from the vulva of one rat into that of another, producing not only a similar tumor but also metastasis. Fuschel tried inoculation experiments, but failed. Klebs, in his inoculation experiments, found that the parts were absorbed. Power[54] assumed that the parasites, as in malaria, were propagated in soil, and collecting soils from districts which were evidently cancer districts impregnated them with cancerous tissue. On these, white rabbits, which had been subjected to irritation on different parts of their bodies were compelled to live for some time. His results were also negative.

Pathologists have succeeded in finding parasites in sarcoma as well as in carcinoma.

Vedeler,[55] after the study of four sarcomas, comes to the conclusion that probably the numerous cell enclosures which he found were Sporozoa. His drawing of the stages of their development compared with those found by Büsse are very significant; and, with the description of yeasts by Corselli and Frisco, cause one to be impressed with the marked similarity of forms which were found by those authors, both in the original tumor and in the tumors produced in the test animals.

Pawlowsky,[56] in his study of fourteen cases of sarcoma, came to the conclusion that the parasites which he found had an etiological connection with the spreading and growth of the tumors. In the description of the life history of the parasites, he maintained that the spore was the starting

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point. His drawings also show strong resemblance to the yeast fungi described by Corselli and Frisco, as well as a similarity in form to those shown by Büsse.

Steinhaus, Hadden, Wernicke, Jackson and Clarke are other pathologists who have recognised similar parasites in sarcomas.

In the author's investigation of tumors the specimens were prepared as follows: Taken in most cases absolutely fresh, and in some still life-warm, they were cut into pieces, five cubic centimeters in size, and placed in a saturated solution of corrosive sublimate at least three hours, but no longer than twenty-four. (The fixing solution was prepared by adding to boiling distilled water as much corrosive sublimate as would dissolve. Upon cooling the supernatant fluid was not decanted, but placed in a dark bottle. The solution will keep only two weeks, and must be placed where it is cool.) After being washed they were removed to 70 per cent. alcohol, where, with daily changes, they remained a week. The strength was then increased daily to 80, 95 and absolute alcohol. Then the specimens were cleared in oil of Bergamot for twenty-four hours, then in xylol twenty-four hours, and finally placed in xylol and paraffine, and paraffine alone. The sections were cut as thin as possible. Besides the stains of eosin and hematoxylin, the Ehrlich-Biondi triple stain, safranine and malachite-green, lithium-carmine and Ehrlich's fluid with gentian-violet were used; but the most beautiful results were derived from the following method: Place the slide for twenty-four hours in a dilute solution of Delafield's hematoxylin (2 per cent. is found strong enough), wash in water twenty-four hours, changing frequently; leave in gentian-violet and aniline-water twenty-four hours, wash thoroughly; differentiate quickly in acid alcohol, then a moment or two in eosin alcohol; dehydrate quickly in absolute alcohol, clear in xylol and mount in balsam.

With a few exceptions, parasites were found in all the specimens examined. These included specimens from eighteen schirrus cancers of the breast, five recurrent cancers

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of the same variety from the same region, two cancers of the penis, one from the upper lip, one schirrus cancer from the mesentery and one from the stomach, one round-celled sarcoma of the stomach and one of the breast. Of these, the results of the research in a few selected tumors will be taken to represent the study of the whole.

In a case of carcinoma of the penis there were seen numerous cell enclosures, which stained similarly to yeast fungi found in cancerous tissue by Sanfelice and Roncali. Some were found in vacuoles and others showed no differentiation from the cell contents but that marked by the stain. A few were found which presented deeply-stained granules. Many of them were so small that they did not change the contour of the nucleus, while a few were of such proportions that the nucleus was not only crowded to the periphery of the cell but was pressed into a crescent shape. Besides those bodies which appeared as real parasites there were found numerous other bodies of forms similar to those described by some of the authors mentioned above. Among these forms were several specimens similar to the so-called *Rhodalocephalus Carcinomatosum*, of Koretneff, which bodies, from the appearance presented in the sections of the writer, could be more readily explained as products of degeneration than as parasites. Steinhaus has described the process by which the cells are gradually pressed together until they become degenerated masses, which upon section may present many different shapes that might be taken for parasites. In this case of carcinoma of the penis the arrangement of the cells about these masses suggested the existence of pressure, the cells in contact with the mass were very thin, much like the superficial cells of the epidermis, there remaining only the evident outline of the cell, without a nucleus. The next layers of cells were thicker, and many of these possessed distorted nuclei, and finally, toward the periphery, perfectly normal cells were seen. Bodies were found which corresponded very closely to some of those described and drawn by Foà, which seemed more like cell invaginations.

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Many parasites were found in the schirrus carcinoma of the breast. These were similar in form and size to those drawn by the various authors who have described the yeasts.

In a case of schirrus carcinoma of the stomach and the metastatic tumors from the axilla, parasites were found, which were identical in form, size and in their reaction to staining reagents. They were uniformly round, homogeneously stained and situated within vacuoles. In a case of a round-celled sarcoma of the stomach, besides forms similar to those mentioned above, there was found a form which was undergoing what seemed to be a process of budding, being similar to those forms described by Roncali in his research.

In comparing the yeast fungi and the parasitic bodies of malignant tumors, something ought to be said in regard to the staining reactions of the latter. Opponents of the parasitic theory claim that all of the appearances presented by the so-called Protozoa can be explained by the staining reactions of the different kinds of cell metamorphoses, as hyaline, colloid, gelatinous and horny degenerations; or by irregularities of cell life, as cell invagination, vacuolisation, dropsy of cell bodies, irregularities in the distribution of chromatin and dispersion of chromosomes, fragments of such during and after the karyokinetic process and asymmetrical mitosis, or they can be explained by the inter- and intracellular invasion of leucocytes and red blood corpuscles. While their statements may be true in the case of many forms which has been presented as parasites—for, at one time or another, no doubt, every one of the irregular forms mentioned above have been pictured among parasites—yet a greater part of them cannot be explained away by any method of staining. In fact, our knowledge of the reactions of the different products of degeneration toward stains is so meager that neither side can disprove claims of the other in many cases. A specific stain has not been found for Protozoa or yeast fungi, so that staining reactions are of little value in procuring evidence in support in either class of bodies.

Most of the forms, as we have seen, which have been

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described by investigators as cancer bodies, can as readily be considered as yeast fungi. As regards the remaining forms, it may be said that, in order to obtain the knowledge that they were dealing with Protozoa, they were compelled to hunt for forms which were not present, and consequently christened as such bodies which were not parasitic.

Finally, in drawing our conclusions as to the relationship of these fungi to the malignant tumors of man, we may say that:

First—From a histological standpoint, we probably have as evidence the results of the investigations of those who, for the past eight years, have upheld the parasitic theory of malignant tumors, men who have seen yeast fungi, but have incorrectly called them Protozoa.

Second—From the researches of Sanfelice, Corselli and Frisco, and Büsse, that yeast fungi have been found in man and the lower animals affected with malignant tumors. These, isolated, grown on nutrient media and inoculated into other animals gave rise to malignant tumors, which showed the same characteristics as the original tumors.

The field of research is but just opened and the investigations confined to a few centers of learning; still, with the main obstacle overthrown—namely, compliance with the laws of Koch, it seems that we are much nearer to the solution of that difficult problem, the etiology of malignant growths.

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A STUDY OF THE CELLULAR PATHOLOGY OF CARCINOMA.

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It is not the intention of the writer, in the following paper, to enter at all into a general discussion as to the etiology of cancer. To do so, in the present state of our knowledge, would be simply to theorise, to indulge in useless speculation, adding rather to the mist of obscurity that enshrouds the origin of these growths than being of any practical or suggestive value.

This mist of obscurity, however, it may be said, is no denser, the veil no thicker than that which up to a comparatively recent period enveloped a number of the diseases with which mankind is afflicted, diseases which we now know to depend upon the presence of a specific organism. One after another of these so-called "microbic affections" have yielded, in so far as the causative agent is concerned, to the careful, patient investigations of the pathologist. There are still many, however, that remain refractory, refusing as it were to disclose the secret of their birth; and among them, overshadowing all perhaps, in the matter of importance to human kind is the one now under consideration—carcinoma.

The genesis of cancer is no longer in doubt; the studies of Waldeyer and of Thiersch having demonstrated almost beyond the possibility of question that it involves that of epithelium generally. Aimless, purposeless, destructive growths then, the proliferative and infiltrating elements of which are epithelial cells—cells always derived from preëxisting epithelium, standing invariably in a direct genetic relation to tissues originating in the epiblastic or hypoblastic layers of the blastoderm.

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In so far as this, our knowledge of the pathology of cancer may be said to be accurate; but here it practically ends. The question now is, and the answer is all important: What furnishes the stimulus for this aimless, infiltrative growth of epithelial elements? What causes these tissue cells to leave their normal situation and by a process of excessive multiplication and proliferation not only to force their way into the lymph-spaces of adjacent structures, but to reproduce themselves in even remote parts of the organism?

Theories without number have from time to time been advanced, our works on pathological science teeming with them, admitting, the while, that they are but theories. Thus long-continued mechanical irritation has been assigned as a cause;—the failure of an organ, like the female breast, to perform its physiological functions;—developmental faults, as the aberrant embryonic remains of Cohnheim;—errors in nutrition dependent on trophic neuroses;—loss of balance, in after-life, between the connective and epithelial tissues, with insubordination of the latter (Thiersch); all of these and a host of others have been assigned a place among the etiological factors, and in a certain way, perhaps, they may all be entitled to such a place. For example, long-continued mechanical irritation is unquestionably, in very many cases, associated with cancer; as witness the often quoted scrotal cancer of the sweeps, the arm of the paraffin worker and the cancer of the lip in smokers of clay pipes. In thousands of such cases, however, of prolonged irritation no cancer appears, and, *per contra*, in hosts of cases of cancer no history of such irritation can be obtained.

In view of these last considerations, we are certainly justified, for the present at least, in holding to the opinion of Cohnheim; that while most if not all of the factors mentioned have a place in the pathology of carcinoma, they tend rather to produce a constitutional, or it may be a local predisposition to the disease than to act, *per se*, as primary or proliferative causes; that a certain weakening or diminution of the normal physiological resistance of the tissues is produced,

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whereby morbid influences, at other times wholly inoperative, are enabled to produce their characteristic effects.

Billroth rather aptly defines this vague "predisposition" as a "specific formative irritability" of the tissues; while the acting morbid influence is referred to as a "specific formative stimulus." Adopting, then, Billroth's terminology (*The Mutual Action of the Living Animal and Vegetable Cell. New Sydenham Soc.*, 1894,) as the more explicit of the two, it would seem as though we are warranted, as has been said, in considering that the various forms of irritation, *et al.*, act by producing a "specific formative irritability" rather than as "specific formative stimuli." And yet for the production in various and varied situations of neoplastic formations as specific and unvarying in type as cancer, there must exist a form of stimulus, and a far-reaching and a continuously-acting stimulus that cannot be considered as otherwise than specific.

There are some features that characterise the growth of a carcinomatous tumor that seem to point to an analogy between them and certain other tumor formations that were some years ago classed by Virchow as "infective granulomata." Thus, that which was said by Dr. Green of a tuberculous deposit may with slight modification be equally well said of a cancer nodule: "The progressive character of the growth and its tendency to infect adjacent and distant portions of the body show the existence of some irritant capable of multiplying in the body and of spreading from primary to secondary foci."—Green, *Pathology and Morbid Anatomy. Seventh Am. ed., p. 554.*

This resemblance to a tuberculous process, this tendency to spread from primary to secondary foci, taken in connection with the characteristic cachexia that marks the progress of a carcinomatous growth, has led to the belief, now more or less widely spread, that cancer itself is of an infectious nature, even though its inoculability from man to man be not absolutely proven. Add to these features a certain observed tendency to hereditary transmission, if not of the disease itself, to at least a predisposition to the disease, and it is lit-

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tle to be wondered at that the belief in an infective or parasitic origin should have become, in the minds of some pathologists at least, quite firmly implanted.

This idea having taken root, it is hardly necessary to state that a most active search for the infective agent has for a number of years been carried on ; with results that are certainly interesting, not to say startling, even while they astonish one by their variety.

Inasmuch as this paper has to do with some of the appearances that in a measure give color to this theory of parasitism in cancer, it may be well to state here that the bibliography upon the subject is already enormous ; so much so that any extensive reference to it is unnecessary and in a limited space impossible. With the exception, then, of a few casual allusions, the writer will content himself with a more or less imperfect demonstration of what may be found here at home in specimens of cancer met with in ordinary routine work.

In 1888, it will be remembered that Neisser, of gonococcus fame, published an elaborate paper embodying the results of his investigations into this subject, and placing the "parasite," which he described, in the *Coccidia* group of the Sporozoa. (*Vierteljahrschrift für Dermatologie und Syphilis*, 1888.) A little later (*British Medical Journal*, December 13, 1890,) Dr. W. E. Russell, of the Royal Infirmary, Edinburgh, described and depicted a so-called parasitic organism, presenting the appearance of round, homogeneous bodies, staining deeply with basic fuchsin and retaining it strongly when treated with other reagents, such as iodine-green. These he denominated "fuchsin bodies," considering them of a fungoid nature.

Contemporaneously with these, and continuing up to the present time, a host of other observers have described microscopic appearances attributed to result from the presence of an organism ; some considering it to be a Fungus or Alga, others a Protozoon. Thus, Soudakewitch, Pio Foà, Thoma, Metchnikoff, Korotneff, G. Sims Woodhead, Ruffer, in conjunction with Walker and Plimmer, C. H. Castle and a host

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of others have, from time to time, described appearances that, whether dependent upon the presence of a parasite or not, demand, from the very importance of the subject, the closest study on the part of every student of pathology.

The assumption, on the part of these observers, that we are dealing with a parasitic organism, has, as is to be expected, met with a storm of criticism from pathologists of the highest repute. Thus, Klebs, Cornil, and, in our own country, Welch, of Johns Hopkins University, Prudden, of New York, and others, while admitting the appearances question the probability of their being due to a parasite, and ascribe them to various sources, to which we will refer later. Looking, however, at the matter from both points of view, weighing the evidence as we get it from the literature, *pro* and *con*, and it becomes at once manifest that we are not by any means justified in taking too radical a stand upon this subject; that there must be something to warrant the opinion of such men as Pio Foà, Thoma, Russell, Ruffer and G. Sims Woodhead, and, such being the case, careful and systematic study is the course that should be pursued, rather than the maintenance of a position of sceptical inactivity.

The feature that strikes one most forcibly, on looking over the various articles upon this subject, is the lack of uniformity in the results obtained. And it is this very feature perhaps that more than any other leads conservative minds to maintain a sceptical attitude, and it was this feature that led, in part, to the series of investigations that form the basis of this article; one of the objects having been to ascertain what there was in the microscopic appearances of cancer that should lead Dr. Russell to describe a round, homogeneous "fuchsin body" as a Fungus, while Neisser described a Protozoon; albeit the boundary line between the two is by no means well defined.

In addition to this, the writer, in making these investigations, hoped to be able to satisfy at least himself as to the following points:

1. Whether in the specimens of cancer met with in ordi-

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nary class-room and routine work, without special methods of hardening and fixing, microscopical appearances could be found that might be interpreted as indicating the presence of an organism or body foreign to the tissues.

2. Whether these appearances are to be demonstrated in *all* specimens of carcinoma.

3. Whether they are peculiar to the carcinomata alone.

The possibility of being able to definitely determine the nature of these bodies was also considered, but it must be admitted, scarcely hoped for.

In selecting the material for these studies it will suffice to say that, with a few exceptions, it was taken from the ordinary material provided for laboratory work in the Medical Department of Yale University, under the supervision of Professor M. C. White. A few specimens, however, were personally prepared by the writer from tissues derived from various sources.

As to methods of fixing and hardening, they were various; chiefly, however, a simple passing of the tissues through alcohols of progressive strengths; as, for example, 55 per cent, 67 per cent., 75 per cent., 82 per cent. and finally into 95 per cent., in which the specimens were kept. One or two specimens, involving nervous tissues, were hardened in "Müller's fluid, while one or two were fixed in a saturated solution of corrosive mercuric chloride for an hour, thence passing through progressive strengths of alcohol, as above. It will thus be seen that "especial methods of fixing and hardening" can hardly be said to be a predominant factor in the production of the appearances about to be described.

With regard to embedding and cutting of sections, all of the methods in common use were employed; thus, some were embedded and cut in paraffin, some in celloidin and some were cut by the aid of the freezing microtome.

As to the methods of staining, a few words may well be said, although they will be made as few as possible.

Inasmuch as the first studies were devoted to the "fuchsin bodies" of Dr. Russell, the methods of staining employed

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by him were naturally first made use of. He, it will be remembered, stained his sections first in a 2 per cent. solution of basic fuchsin in 5 per cent. carbolic acid; they were then placed in a 1 per cent. solution of iodine-green (Grübler's) which replaced the fuchsin in everything but the above mentioned bodies. (Op. cit.)

The writer soon found that practically the same or better results could be obtained with fuchsin and methylene-blue; there being no difficulty in demonstrating, in many sections of carcinoma, the rather characteristic, deeply stained, homogeneous bodies referred to. They vary in size from four to eleven or twelve micro-millimeters in diameter, and are met with singly, in twos, or threes, or in clumps of four or five lying both within the protoplasm of the cells and in the intercellular spaces. They are not confined, however, to the carcinomata for a section of lympho-sarcoma now at hand shows them in large numbers.

Not wishing at this time to devote much space to these bodies the writer would suggest, in taking leave of them, that they certainly merit close study. Red blood corpuscles readily take up and strongly retain basic fuchsin; and there are certainly in some of these "fuchsin bodies" appearances that suggest red corpuscles. There is, however, rarely so much variation in the size of the corpuscles, and they are almost never found within the cell protoplasm, although the writer has in one or two instances so found them.

Again the variation in the size of the "fuchsin bodies" serves as no real basis of distinction. As is well known we may have in certain pathological conditions the greatest diversity in the size and appearance of the red corpuscles. Thus in chlorotic conditions and pernicious anemia the so-called *microcytes* and *macrocytes*, as well as the huge "giganto-blasts" of Eichhorst, twenty micro-millimeters in diameter, are of frequent occurrence. For all this the writer, after careful study, is not disposed, for many reasons, to regard these "fuchsin bodies" as red blood corpuscles; their precise nature, however, it is impossible at present to state.

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While still engaged in the study of these bodies, with other reagents than methylene-blue and fuchsin, certain appearances presented themselves from time to time, in some sections of carcinoma, that were not explainable by ordinary methods of reasoning. For instance in staining with such combinations as hematoxylin and safranine, hematoxylin and acid fuchsin, and hematoxylin and eosin there appeared to be certain enclosures in some of the larger epithelial elements that differed in appearance from the cell nuclei, and also from the bodies previously described; and yet the differentiation was not sufficiently well marked, both nuclei and enclosures staining with hematoxylin, to warrant an expression of opinion as to their nature.

Without going too much into detail it will suffice to say that in the attempt to further differentiate these enclosures there was no staining reagent, or combination of stains in common use, but that was made use of, with, however, but indifferent success. At length attention was directed to an article or paragraph in Landois' Physiology (4th ed., p. 389,) on the beautiful results to be obtained in the demonstration of the nuclear figures that appear during the process of mitotic or indirect cell division (karyokinesis) by the use of a reagent now widely known as the "Ehrlich-Biondi" triple stain.

A trial of this reagent soon showed that by its use the enclosures mentioned above, the cell nuclei, and in fact, all of the component parts of the tissues could be differentiated with almost diagrammatic effect.

This "Ehrlich-Biondi fluid," as it was formerly called, owes its introduction really to Haidenhain; and is a mixture of methyl-green, methyl-orange or orange G. (Grübler), and acid fuchsin; (Rubin S. "saure fuchsin"). It is prepared by Grübler in a dry form ready mixed for use, 100 cubic centimeters of a 0.4 per cent. solution, with the addition of 7 cubic centimeters of a 0.5 per cent. solution of acid fuchsin (both aqueous) making the ordinary desk reagent. The method of using is given in nearly every recent work on histology.

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Staining with this reagent *secundem artem*, using thin sections, cut in paraffin and with careful attention to technique we are told to expect the following results: the nuclei to appear of a bright green, the cell protoplasm taking an orange red or a reddish orange, while the connective tissues appear of the vivid or crimson red of the fuchsin.

Briefly, while we expect these results we don't always get them. At the best the stain is an uncertain one, acting at times and with certain tissues in a most inexplicable way. In this the experience of the writer coincides, I think, with that of most microscopists. At the same time, with all its uncertainties, it is a most invaluable aid in histological studies; a well stained section presenting a beautiful picture, and one that as has been said is almost diagrammatic.

Attention may be called to the fact that certain methods of hardening are not compatible with the use of this stain; thus in sections hardened in "Müller's fluid" the cell nuclei appear of a peculiar blue color, while the protoplasm appears of a rosy red, lacking, however, entirely in orange. Again embedding in paraffin seems to produce the only perfect results, inasmuch as sections cut in celloidin rarely show any orange in the protoplasm, unless it be after excessively long exposure to the stain. Embedding in paraffin, however, in itself leaves, in many cases, much to be desired. In tissues particularly of an encephaloid or purely cellular type, a marked and detrimental shrinkage is sure to take place. With celloidin this does not occur; and in such tissues it is vastly to be preferred. The slight differences in staining incurred by its use, are after all but differences of degree; and to one at all practised in histological work in no way impair the usefulness of either the reagent or method of embedding.

Returning now to the enclosures that have been referred to as occurring in certain of the epithelial cells of some sections of cancer, we may consider the appearances produced by the use of this "Biondi" reagent.

These, in the main, do not differ from those above

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described; but there appear in many of the cells, bodies that stain differently, and in other ways present an appearance entirely unique.

These enclosures appear to be of two kinds. Thus some of them appear to possess a distinct capsule or wall of a vivid red color, a protoplasm that remains colorless and a small distinct nucleus either centrally or laterally placed and which is also of a bright red. They are almost invariably contained within the cell protoplasm, it being very unusual to find them extra-cellular in position. They are found singly, in groups of two and three, or more, while in some cases the cell appears crowded with them, the nucleus being displaced or, as occurred in one instance, having entirely disappeared. In size they vary from scarcely more than two or three micro-millimeters in diameter up to twelve, or possibly fifteen or even more.

The other form presents an appearance entirely distinctive from this. In the first place they are never so small, rarely falling below twelve micro-millimeters in diameter. They are also perfectly spherical, have a small distinct nucleus, but the protoplasm stains most decidedly; appearing of an indescribable lilac color, or of even a distinct bluish red; the nucleus itself being of a more decided red. They are rarely found otherwise than one in a cell, although two cells containing them may be closely approximated. They never appear in groups.

Reference to the accompanying plates will give a better idea of these enclosures than could be obtained from pages of text.

The sections from which these drawings were made were taken from perhaps six or eight selected specimens of carcinoma, including both primary and secondary growths. All of these, I think, with one exception, were removed by Professor W. H. Carmalt, at the New Haven hospital, during the past year. This exception, and the one most prolific in the matter of "enclosures," was a secondary growth of an "encephaloid," or purely cellular type, involving the cere-

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bellum and resulting in death. The primary growth was a scirrhus cancer (fibro-carcinoma) of the breast, which was also removed by Professor Carmalt some two years before.

The results embodied in these plates, with the brief appended description, may be said to be the results of something more than a year's study into the microscopical appearances of the carcinomata. Sarcomatous tissues have also come in for their share of study, with results that, while of great interest, are as yet too imperfect to warrant a consideration of them at this time, even if space permitted.

As to the "cancer bodies," or enclosures, the writer simply presents them as evidence corroborative of the results obtained by other pathologists. As will be noted, they correspond, except in minor details dependent in part upon a difference in "technique" in preparation, to the bodies described by, at least, Ruffer and Pio Foà. The results obtained by other observers are strangely at variance, and, it must be admitted, unexplainably so.

For a discussion, *pro* and *con*, as to the nature of these bodies, it is sufficient to refer to *Sajous' Annual of the Universal Medical Sciences* for 1895.

With regard to Cornil's idea that they are simply invaginated cells, it is hardly to be thought of when we refer to the examples of this depicted in Plate I., Figs. 4, 5 and 6, and Plate III., Fig. 24. Dr. Welch, of Johns Hopkins, in expressing the opinion* that they are fragments of eleidin or keratohyalin, or other products of protoplasmic degeneration, is probably in error. If such were the case, fragments of eleidin or kerato-hyalin, under similar treatment, would present the same appearances *in normal tissues*, and the same may be said regarding other explanations that have from time to time been offered.

A process of endogenous cell formation has been suggested as explanatory of these appearances; but here again comes in "control sections" of other instances of exceedingly rapid cell proliferation, such as granulation tissue and certain forms

* Quoted by Dr. Ruffer, *Jour. Path and Bact.*, 1893.

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of rapidly growing adenomata. Such appearances, arising from endogenous cell formation, or any other method of cell formation are not, and, so far as the writer's knowledge goes, never have been demonstrated in these or other tissues.

Mastzellen, the so-called "feeding cells" of Altman, have also been mentioned in this connection, for no other reason that the writer can see than that certain of the Protozoa in their early stages present the appearance of a simple mass of granular protoplasm. These *Mastzellen* are very abundant in certain rapidly growing tissues, as, for instance, in young animals, such as the sheep, where they may be readily demonstrated in the lung. They are also met with very frequently in the human subject, and are particularly noticeable in some sarcomata.*

Professor Klebs, in June, 1890, refers to these enclosures as hyaline bodies, and is "decidedly disposed to regard them as degenerative products" (*Deutsche Medicinische Wochenschrift*, Nos. 24, 25, 32, June, 1890). Without, however, going into the discussion further, it may be said that the foregoing sum up the most rational attempts at explanation of these appearances, aside from those in which they are regarded as Protozoa.

In so far as the writer's experience goes, these enclosures, *as above described*, are peculiar to the carcinomata. Professor M. C. White states that he has met with one or more in a section of apparently normal kidney. The writer, however, had no opportunity of examining it. A. A. Kanthack says (*British Medical Journal*, March 14, 1891,): "These organisms, whether we call them Protozoa, or psorospermiæ, or sporocysts, or what not, occur, and even frequently, in other diseases, even in apparently healthy tissues." Be this as it may, the study by the writer of over a thousand sections of various tissues has failed to show them in any other situation.

As to their occurrence in *all* carcinomata as much can not be said. Some sections have failed to show them, but sec-

* See Plate I., Fig. 9.

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tions from another portion of the tumor, possibly, might have done so. In the vast majority of instances, however, they are to be found without great difficulty. They are more abundant, seemingly, in soft, rapidly growing cancers, as for instance, the encephaloid growth above referred to. They are also more readily demonstrated, apparently, in secondary growths, although this cannot be said to be invariable. They cannot be said to be everywhere present, or, as a rule, abundant. One may have quite a search without finding a single enclosure, while again a solitary field may show half a dozen or more. As has been said, they are almost invariably contained within the protoplasm of the cell. The writer has never found one within the cell-nucleus, and in but one or two instances have they appeared outside the cell.

A short time since, the writer was enabled, through the courtesy of Dr. Charles J. Foote, to see the colored plates, together with original paper, of Ruffer and Walker (*Jour. of Path. and Bact.*, 1893). Engravings, however, prepared from these, appear in Green's *Pathology* and *Morbid Anatomy* (Seventh Am. Ed.).

Dr. Ruffer employed the "Ehrlich-Biondi" stain with good effect, describing, however, an orange cell protoplasm with a parasite (?) of "Cambridge" blue. Precisely his effects the writer has been unable to obtain, but it is to be noted that he embedded and cut in paraffin, while the drawings that accompany this paper were from tissues cut in celloidin. Again, Dr. Ruffer describes the parasite as refractory to hematoxylin, while Pio Foà (*Annual Universal Medical Sciences*, 1894,) demonstrated his bodies most satisfactorily by its use. The writer finds, with him, that they stain very readily with ripe hematoxylin (Gage's), but inasmuch as the cell nuclei do the same, it requires a practised eye to differentiate them.

We have thus, in a necessarily brief and incomplete way, studied some of the appearances that, as has been said, in a measure tend to give color to the theory that cancer is dependent upon the presence and growth of a specific organ-

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ism; and we have also considered, in part, the various explanations that have from time to time been offered to account for these appearances. Most, if not all, of these attempts at explanation, however, are manifestly inadequate and by no means cover the entire ground, and hence it is useless, in the face of such a mass of evidence as has accumulated, to attempt to deny that the cells of carcinoma, in many cases, contain structures that, if we can rely upon our present teachings in the science of histology, are not an integral portion of the tissues. At the same time, however, we are forced to admit that evidence as to their precise nature is wholly and entirely wanting. And this must be so, inasmuch as at present we are dependent upon mere morphological appearances for our knowledge, rather than upon biological detail, and a knowledge of the life history of the organism, if organism it be. Artificial cultures have been attempted, but up to the present time have not been successful. We are hence compelled, as it were, to suspend judgment, bending our energies meanwhile to the development of thorough and systematic methods of study, methods which involve the study of the living, growing cancer, rather than that of simple microscopic sections alone.

The fact has been before referred to that Neisser (*loc. cit.*) was among the first to regard these structures as Protozoa (class Sporozoa, sub-class Coccidia). In this he is supported by nearly all of the recent observers, including that most accurate of zoologists, Metchnikoff. Even the great Billroth inclines to the same belief (*op. cit.*). He was apparently much impressed by the investigations of Bollinger and J. Pfeiffer into the etiology and pathogenesis of the infectious epithelioma of birds; they stating as follows: "The epithelioma formation is brought about by the immigration of the germs of a Protozoon of the class of the Sporozoa (*Gregarinæ*) into the cells of the *rete malpighii*. Whilst the cells affected by the parasite are entirely consumed up to a narrow border line, and the nucleus pressed against the cell wall, a proliferation of the still intact epithelial cells in the neighborhood of

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the invaded region takes place, the progeny of which are successfully invaded by the parasite."

The simple comparison of this description with some of the appearances in the accompanying drawings* in itself furnishes some food for thought.

For the best brief exposition of the life history of the Protozoa themselves, the reader, perhaps, had better refer to Lankester's article in the *Encyclopedia Brittanica.* A few words, however, based upon his terminology may perhaps be offered as a fitting conclusion to this paper.

"Protozoa," as is well known, is the name applied to the lowest grade of the animal kingdom, being sharply and definitely distinguished from the higher groups of Metazoa and Enterozoa by the fact that they are structurally single cells or units of protoplasm; whereas the latter consist of aggregations of such units, which are embryonically arranged into two, or in the highest types, three layers. While, however, the Protozoa are essentially unicellular organisms, this is by no means always the case; as many consist of aggregations of such cells with, however, this marked and peculiar distinction, there is no differentiation into embryonic layers, and each component cell is capable at any time of taking up and maintaining an *independent existence*, the cohesion between the cells having no economic significance.

As distinguishing the Protozoa from the lower grades of plant life—the Fungi and Algæ—we have the one great feature that marks plant life as distinguished from animal life in the higher orders; the plants are synthetical, capable of elaborating their nourishment from the simplest compounds, as carbonic acid and ammonia, while the Protozoa, like other animals, require the preformed, higher organic compounds for their nutrition. (The somewhat anomalous position of some of the Fungi it is unnecessary to discuss here.)

The Protozoon individual, then, is a simple mass of protoplasm, varing in diameter from $\frac{1}{1000}$ of an inch up to an inch (as witness the gigantic *Nummulites*). In common with

*Plates II. and III.

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other forms of animal life, they are endowed with the power of motion, possess a certain irritability, and are capable of growth and reproduction. Perhaps not their least important characteristic, when considered in connection with cancer, is that of "producing by chemical processes that take place in their substance (over and above those merely related to nutrition) a variety of distinct chemical compounds, which may form a deposit in or beyond the superficial protoplasm of the

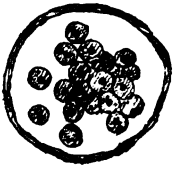

FIG. 28.
Cyst of *Klossia helicina*.

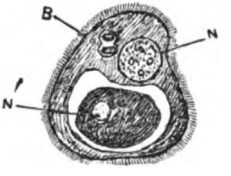

FIG. 29.

Renal cell, from *Helix hortensis*, containing full grown Gregarine, *Klossia helicina*, (copied from Lankester). Compare with Figs. 16, 17, 18 and 26. *N*. Cell nucleus. *N'*. Body, nature unknown. *B*. Nucleus of *Klossia*.

corpuscle, or may accumulate centrally." It will be noted that there is every possibility of these chemical products producing analogous effects to the so-called "toxines" of the pathogenic bacteria.

As to modes of growth and reproduction, the Protozoon follows the same course as tissue cells in general. While simple binary division is the rule, it is very usual that under given conditions the Protozoon breaks up rapidly in from ten to a hundred little pieces, each of which leads an independent life and grows to the form and size of its parent. This is shown in Fig. 28, where the Protozoon has gone on to the

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formation of a cyst, the contents of which break up into a number of *chlamydospores* (coated spores). The analogy to Figs. 15 and 23 is very striking.

A discussion as to the part played by various Protozoa in the life drama of both vertebrates and invertebrates, while of exceeding interest, is impossible at this time. As is well known, they are intimately associated in the human family with Paget's disease of the nipple, with the so-called *Keratosis follicularis*," and with *Molluscum contagiosum.*" The often quoted *Coccidium oviforme*, that so frequently occurs in the liver of the rabbit, particularly of those that inhabit marshy districts, not infrequently gains access to and excites inflammatory processes in the human liver. The so-called "Rainey's tubes," or "sacs of Meischer," that occur in the striated muscle fibers of the hog (*Sus scrofa*), are simply the chlamydospore cysts of a Protozoon, in the majority of cases being filled with falciform young. The epithelioma of birds we have already referred to. Finally, as we pass down the animal scale, the gills of the fresh water perch, the common earth-worm, the garden snail and the familiar cockroach (*Blatta orientalis*) are all of them fertile fields for the demonstration of Protozoa.

These instances, together with innumerable others that might be cited, simply show the extent to which these lowly organisms participate in pathological as well as apparently non-pathological processes occurring in the various types of animal life.

In concluding, the writer once more begs to call attention to the fact that the foregoing demonstration of the cell-enclosures of cancer simply corroborates the results obtained by a number of pathologists during the past hemidecade. In no sense do they furnish indubitable evidence that the disease is dependent upon the presence of a parasite. Neither can there be said to exist even proof positive that these bodies are Protozoa. The resemblance, however, of the anatomical details here presented, to those that illustrate the various phases in the life history of certain of these

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organisms, is certainly very striking. Still, the evidence is far from complete, and for the present we are simply justified in considering the whole matter as *sub judice*. In the face, however, of the facts presented, we can certainly do no less than admit that the cells of carcinoma, in very many instances, present appearances that are wholly and entirely unexplainable by any reasoning based upon our present knowledge of pathological histology.

The writer wishes, in closing, to acknowledge his great indebtedness to Dr. M. C. White, Professor of Pathology in Yale University, for the advice and assistance so kindly and courteously given.

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PLATE I.

Fig. 1. Cancer cells.
n. Nuclei.
s. Small round cells.

Fig. 2. Cancer cell.
Nucleus dividing.

Fig. 3. Cancer cell.
Two nuclei.

Fig. 4. Cancer cells.
Invaginated. *n*. Nuclei.

Fig. 5. Cancer cells.
Invaginated.

Fig 6. Cancer cells.
Division after invagination.

Fig. 7. Cancer cell.
Hydrophic degeneration about nucleus (*n*).

Fig. 8. Cancer cells.
Invaginated cell; nucleus broken up.

Fig. 9. Mast Zellen. (Methyl-blue.)

Zeiss. Objective, $\frac{1}{12}$. Oil immersion. Ocular, 4. C. W. K., Pinx.

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Plate I.

Fig 1.

Fig 2.

Fig 3

Fig 4

Fig 5.

Fig 7.

Fig 8

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PLATE II.

Fig. 10. Cancer body or enclosure.
From scirrhus of breast.
n. Nucleus.

Fig. 11. Cancer body or enclosure.
(From another case.)

Fig. 12. Two cancer bodies in one cell.
(From scirrhus.)

Fig. 13. Three cancer bodies.
(From scirrhus of breast.)

Fig. 14. Five cancer bodies involving two cells.
From encephaloid of cerebellum.
v. Vacuole.

Fig. 15. A crowd of cancer bodies.
From same specimen as Fig. 14.

Fig. 16. Different type of enclosure.
From encephaloid.

Fig. 17. Different type of enclosure.
From same growth as in Fig. 16.

Fig. 18. Two enclosures involving two cells.
From same growth as in Figs. 16 and 17.

Zeiss. Objective, $\frac{1}{12}$. Oil immersion. Ocular, 4. C. W. K., Pinx.

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Plate II.

Fig 10.

Fig 11.

Fig 12

Fig 13.

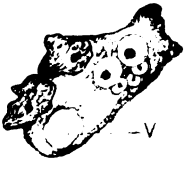

Fig 14.

Fig 15

Fig 16.

Fig 17.

Fig 18.

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PLATE III.

Fig. 19. Two cells.
(From scirrhus of breast.)

Fig. 20. One field.
(From scirrhus.)

Fig. 21. Two cells.
(From scirrhus.)
n = Fragment of nucleus.

Fig. 22. Single cancer alveolus, showing three enclosures.

Fig. 23. Single cell.
Nucleus has disappeared.
(From secondary cancer of liver.)

Fig. 24. One field.
Enclosures and invaginated cell.

Fig. 25. Apparent division.
Nucleus displaced.

Fig. 26. One field.
(From encephaloid.)

Fig. 27. Fuchsin bodies. (Russell.)
Fuchsin and iodine. (Green.)

Zeiss. Objective, $\frac{1}{12}$. Oil immersion. Ocular, 4. C. W. K., Pinx.

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Plate III.

Fig 19.

Fig 20.

Fig 21.

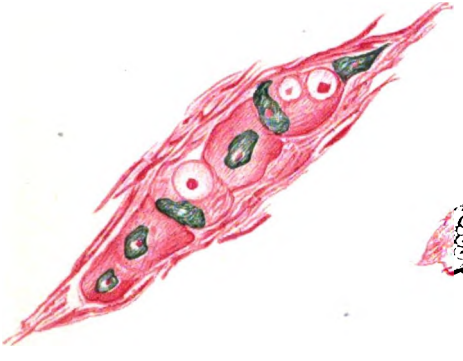

Fig 22

Fig 23.

Fig 24

Fig 25

Fig 26.

Fig 27.

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DIPHTHERIA—ITS BACTERIOLOGY.

CHARLES F. CRAIG, M. D., DANBURY, CONN.

HISTORICAL.

The history of the discovery of bacteria in this disease is one full of interest. In 1868, Oertel found micrococci present in the false membranes, and in investigations made in 1874 he found the *Bacterium termo* present. He was even successful in producing croup experimentally in animals, and stated his belief to be that these micrococci were the cause of the disease. Nasililof, Hueter and Tommasi also found micrococci present in this disease, and Klebs, in 1871, and Eberth, in 1872, claimed that the diphtheria micrococci were identical with those of sepsis. At this time the theory of the microbic origin of this disease seemed well founded, but it had many enemies, among the most prominent of whom were Senator and Billroth.

In 1877, Drs. Curtis and Satterthwaite were selected by the New York City Board of Health to investigate the cause of this disease, and as the result of their investigations they reached the conclusion that the so-called diphtheria micrococci were not essential in the production of the disease, and Drs. Wood and Formad, who conducted a series of investigations for the National Board of Health, arrived at the same conclusions. It was thus that the question stood when, in 1883, Klebs first called attention to a bacillus which he found upon the false membranes, which he cultivated, and in 1884 Löffler published his investigations upon the same organism. He was able to separate and cultivate it, and, though not at that time able to produce the typical disease in animals by inoculation, he showed that the organism was poisonous.

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His observations were confirmed by Fränkel, and Roux was shortly after successful in producing the disease in animals by using cultures of the bacillus. The organism became known as the Klebs-Löffler bacillus, and is now generally recognised as the cause of diphtheria.

Very valuable contributions to our knowledge of this organism have been made by various observers, among the more recent being those of Kolisko and Paltauf, of Vienna; Ortman, of Königsberg; Escherich, in Munich; Bech, Brieger, Fränkel and Behring, of Berlin; Babes, in Bucharest; Klein, of London; Roux and Yersin, of Paris; and Welch, Abbott, Prudden, Park, Councilman and others, of this country.

DESCRIPTION.

The Klebs-Löffler bacilli are short rods, straight, or slightly bent, about as long as the tubercle bacillus, but much thicker, and presenting a plump appearance. The ends of the rods are rounded. These bacilli vary greatly in size and appearance under different modes of cultivation, sometimes appearing to be enclosed within a capsule, either as a straight rod or transversely segmented, or oval or elliptical in shape, with swollen ends. Very often, either at the ends or the middle of the bacilli, small round portions, more deeply stained than the remainder of the bacilli are seen, which by some authorities are regarded as spores, but spore formation has not as yet been proved. They are non-motile. In microscopic slides made from portions of false membrane, the bacilli appear in patches and clumps, and also scattered singly over the field examined. The bacilli are found only upon the false membranes and the mucous membranes of the air-passages. They are very resistant, and have been found to be virulent in dried membrane after four weeks. Roux and Yersin found that serum cultures were virulent, under ordinary conditions, after five months, and if protected from heat and light after thirteen months.

Regarding the persistence of the bacilli in the throat during and after recovery from the disease, Park, the inspector

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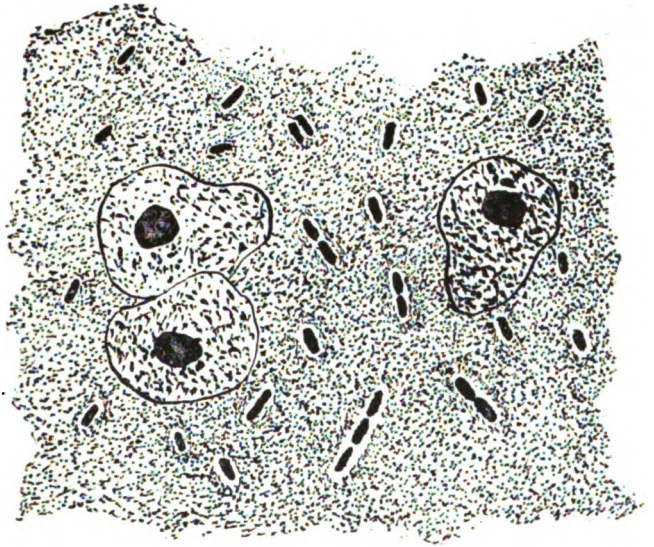
of diphtheria for New York City, reports that of 752 cases examined, 325 were free of bacilli in the throat three days after the disappearance of the membrane, 201 after from five to seven days, 84 after twelve days, 69 after fifteen days, 57 after three weeks, 11 after four weeks, and 5 after five weeks. In one case the bacilli were found after seven weeks, and not only present but virulent.

He also found in healthy persons exposed to the disease, where isolation was not practised, virulent bacilli in 50



+ 1,200 and enlarged threefold.

per cent. of the cases examined, forty-five in number, and that, of these, 40 per cent. later developed the disease. Where isolation was carefully attended to, virulent bacilli were found in only 11 per cent. of the cases examined of healthy persons exposed to the disease.

Park and Beebe examined the throats of some 330 persons in New York City, who, as far as known, had not been exposed to diphtheria, and found virulent bacilli present in eight cases.

Park has cultivated virulent bacilli from dried stains on spreads and bed linen, where diphtheritic patients had expec-

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torated, and has also shown that the sputum of patients, even after being free from bits of membrane, contains great numbers of the bacilli. Wright and Emerson have obtained virulent cultures from the hair, finger nails, shoes and clothing of nurses to diphtheria patients, and also from chairs, brooms and other objects in the diphtheria ward of the Boston City Hospital, and also in dust collected from the same ward. Williams, of Boston, has found that 50 volume solution of hydrogen dioxide will kill the diphtheria bacillus in five seconds in the laboratory, and recommends it in the treatment, locally, of the disease. A solution of 1-4000 bichloride of mercury is much thought of by Park in the local treatment of the disease. The bacilli perish at a temperature between 45° to 50° C. (112° to 122° F.)

STAINING.

These bacilli do not stain by Gram's method, nor well by the ordinary aniline dye solutions. They stain best by the following method. Make up the following staining solution:

Alcohol sol. methyl-blue	30 c. c.
0.01 per cent. sol. caustic potash	100 c. c.

(1 part caustic potash in 10.000 of water.)

Stain the specimens in this for ten minutes, and then place them for fifteen seconds in a ½ per. cent. acetic acid solution. Wash in absolute alcohol and mount in balsam. The bacilli will be stained a dark-blue color.

Crouch, of Denver, in an article recently published,* states that he believes that a direct cover-glass diagnosis of this organism can be generally secured by attention to certain peculiarities in the behavior of this bacillus to certain stains. He found that if a cover-glass prepared as usual, from a blood serum culture, about twenty-four hours old, be treated a few seconds only with methyl-green, in 1 per cent. solution, the following appearances were noticed: "The majority of the bacilli will be stained faintly green and contain at both ends a well-defined round body much more deeply stained, and of a distinctly red color."

* *New York Medical Journal*, October 5, 1895, page 430.

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He found the following fluid most serviceable :

1 per cent. sol. methyl-green (freshly prepared)	5 parts.
1 per cent. sol. dahlia (freshly prepared)	1 part.
Distilled water	4 parts.

He states that only a second is required for staining—staining being too intense after a longer time. He further says : "By smearing a piece of the membrane on the cover-glass, drying and flaming in the usual way, and staining (with the above solution) one or two seconds, the diphtheria bacilli, or certain of them, will present the appearances described above. Wherever I have found such forms, even if only two or three, in the direct cover-glass examination, the cultures have developed diphtheria bacilli without one exception, so that I have come to regard this reaction as of the greatest diagnostic importance."

CULTURE.

These bacilli are facultative anerobic, growing best between 60° and 104° F. They grow on gelatine, potato and other media, but the media must be slightly alkaline in reaction. Kolisko and Paltauf found that growth takes place readily upon nutrient bouillon containing sugar. Löffler obtained the best results upon the following media :

Blood serum (cattle)	3 parts.
Beef bouillon	1 part.
Peptone	1 per cent.
Common salt	½ per cent.
Grape sugar	1 per cent.

They also grow especially well upon glycerine agar. Cultures on this media are dull white in color, flat, and present a glistening appearance, with smooth edges and about the size of a millet seed. They develop in from twenty-four to forty-eight hours. Welch and Abbott have found that the bacillus can be cultivated upon potato. Thrust cultures in gelatine are composed of white globular colonies, which develop along the line of puncture.

In some of the large cities of this country the resident board of health makes cultural examinations of suspected cases,

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for the convenience of physicians, and the methods employed being of interest, I therefore quote largely from a paper by Park,* the inspector of diphtheria in New York City, regarding the manner of collection and examination of the specimens. The methods described can be carried out well by the general practitioner, where there are no laboratory facilities. Following are quotations from his papers:

"*Technique of Preparing the Serum Tubes and the Swabs.*—The blood is received directly from the slaughtered sheep or calf into large, thoroughly cleansed preserve jars, and covered. These jars are put as soon as possible on the ice. After a few hours the jars should be inspected, and if the clot is found adhering to the sides it should be separated. After twenty-four hours on the ice, the serum is poured off and mixed with one-third its quantity of nutrient bouillon, to which 1 per cent. glucose has been added. This is poured into test tubes, which should be about five inches in length and filled one quarter full. They are placed very obliquely in the serum coagulator, and kept just below 100° C. for one hour on two consecutive days The tubes with the sterile solidified blood serum can then be placed in covered tin boxes and kept for months. The serum prepared in this way is quite opaque, but its value is not lessened for the purpose for which it is intended.

"*The Swab.*—A stiff piece of wire, or, better, a thin steel rod, six inches in length, is roughened at one end by a few blows of a hammer. About this a little absorbent cotton is firmly wound. A number of these are placed in an equal number of glass tubes, the ends of which are plugged with cotton. They are sterilised by dry heat at about 150° C. for one hour, and stored for future use.

"For convenience in carrying the tube containing the blood serum and the tube containing the swab, they are wrapped in a little cotton and placed in a cheap, strong pencil box.

* Diphtheria and other Pseudo-Membranous Inflammations. *Medical Record*, February 11, 1893.

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"*Directions for the Physician to Use in Inoculating the Tubes with the Exudate.*—The patient should first be placed in the best available light, and if a child, properly held. Taking the swab from its tube, the tongue is depressed, and the side of the swab is rubbed firmly against any visible membrane, thus catching little particles in its meshes. Without laying it down, it is inserted the full length of the blood serum tube, and the part of the swab which was previously rubbed against the throat is drawn rather firmly along the full length of the serum surface. It is then re-inserted in its own tube for future use in making control cultures.

"A second culture on a blood serum tube is made from the swab (at the laboratory office). Both serum tubes are placed in an incubator at 37° C. for twelve hours. They are then ready for examination. On inspection the blood serum surface will be seen to be dotted with very numerous, just visible, translucent colonies. At this time no diagnosis can be made by simple inspection. He then takes a clean cover-glass and with a platinum loop makes a sweep over a majority of the colonies, and smears upon the cover-glass, staining with the Löffler methyl-blue, as described under *Staining*. There will then be seen either a large number of characteristic diphtheria bacilli, with a small number of diplo- or streptococci, or a more equal distribution of the true bacilli and the other cocci, or else, where diphtheria is not present, a pure culture of diplococci or streptococci."

Regarding the diagnostic value of this method, he says: "A very extended trial has convinced me that cultures on blood serum, made immediately from the fresh exudate on sterile swabs, can be thoroughly relied upon to show a growth of Löffler bacilli, when these were present and living in the throat at the time of the swabbing, whether visible membrane existed or not."

The method above described can be carried out by the general practitioner, and as a reliable diagnosis of diphtheria is many times of inestimable value in protecting other lives, it should be done where access can not be had to a bacterio-

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logical laboratory, and where doubt exists as to the nature of a given case. The general practitioner should be something of a bacteriologist, for many lives often depend upon his diagnosis of just these cases.

EXPERIMENTAL.

Inoculations in animals of cultures are not always followed by success, rabbits, guinea-pigs, chickens and doves being very susceptible, rats and mice not very much so. In the susceptible animals, false membrane is formed in the trachea and sometimes there are constitutional symptoms. Guinea-pigs are very susceptible, and die in a few days after inoculation.

Babes, in a series of experiments, irritated slightly the conjunctivæ of rabbits and then placed upon them pure cultures of the bacillus. The rabbits died in a short time afterward.

Brieger and Fränkel have succeeded in separating a tox-albumin from cultures of the bacilli, which when injected produces albuminuria and paralysis.

IMMUNITY.

Fränkel, Brieger, Kitasato, Behring, Roux and other observers have studied the problem of producing immunity to this disease in animals. Behring and Kitasato have done so by making use of sterilised cultures, by adding iodoform to cultures, and in various other ways, and they found that animals rendered immune are not only safe from the living diphtheria bacilli, but also from the products of their metabolism. Fränkel also obtained immunity by using sterilised cultures.

ANTITOXIN OF DIPHTHERIA.

Of great practical interest is the question of the treatment of this disease by its so-called antitoxin. The principle involved is that the blood serum of an animal rendered immune to the disease, is possessed of curative power when injected into a patient suffering from the disease, by virtue of

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an antitoxic substance which has been elaborated. The method of preparing the antitoxin depends upon continuously and progressively injecting a very powerful toxin into an animal ordinarily non-susceptible (the horse is used), and thus the production in this animal's blood of the antitoxin. The antitoxin is in the serum only of the blood.

In 1888, Roux and Yersin discovered and isolated the diphtheria toxine, and to Behring, of Berlin, belongs the credit of the great discovery that the blood of an animal immunised to this disease may be used in treating the disease in the human subject. His observations were published in 1894, and since that time the treatment has been followed out in a great number of cases, both abroad and in this country. In the *Berliner Klinische Wochenschrift*, 1894, No. 36, Behring thus sums up the blood serum therapeutic method:

"1. It is an antitoxic method by which we endeavor to combat those infectious diseases which we know to be of micro-parasitic origin. These include the infectious diseases and certain vegetable and animal poisons (as snake poison). The specific antitoxins, which are the active agents, have until now been found in quantities sufficient to be available for human medication only in the blood of immunised animals.

"2. It is a principle of the blood serum therapy that larger doses are never injurious, but, on the contrary, can be only beneficial.

"3. The blood serum therapy is a specific therapy. Each blood antitoxin is immunising and curative only for one infection.

"4. Under the influence of a specific toxin there is produced a specific antitoxin from the albumin of the living cell. Whilst this is going on there is a disturbance of the regulating mechanism of the general organism. The febrile and other symptoms of a toxic infection are an expression of the effort of the living organism to render the foreign poison innocuous. In animal experiments we can so arrange things that the living organism succeeds. In immunising animals

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we render the absorption of even larger quantities of the poison harmless by increasing the antitoxin production.

"5. If we examine the bodily fluids after recovery from an artificial or natural toxic infection, we find not only that the toxin is compensated by the antitoxin, but that there is a surplus of the latter. This surplus is the reason why a larger quanity of the toxin must now be introduced in order to produce intoxication. And this surplus can be employed to help other individuals to overcome the same intoxication. The entire blood serum therapy rests on this fact.

"6. Since these antitoxins are soluble chemical bodies, it is not impossible that they may eventually be produced outside the living body, or even compounded synthetically."

This treatment, as applied to diphtheria, has been elaborated by Aronson, Roux and Yersin, and others, and today stands preëminent as a life-saving measure in this disease. It has received the endorsement of such eminent clinicians as Baginsky, Virchow, Ganghofer, Sonnenberg, Kolisko, Bokai, Moisard, Fischer, Welch, Park, and many others, and should always be tried along with the older methods of combating the disease. In 9,487 cases, tabulated from reports in the *Medical News*, 1894–95, and *Bulletin of Johns Hopkins Hospital*, July–August, 1895, the death-rate is 16.5 per cent. where antitoxin serum was used, while by the old methods alone the death-rate was 47.5 per cent. In the *Deutsche Med. Wochenschrift* (1895, No. 32,) is published a collective investigation of cases treated both with and without the antitoxic serum. In Berlin, of 562 cases treated by serum, 84 died, or 15.1 per cent.; of 282 cases treated without the serum 49 died, or 17.4 per cent. Outside of Berlin, of 5,271 cases treated with the serum 9 per cent. died; and of 4,197 cases treated without the serum 14.4 per cent. died, Totals, 5,833 with serum, 9.6 per cent. died; 4,479 without serum, 14.7 per cent. died. From the foregoing reports, it will be seen that the mortality in this dread disease has been reduced nearly or quite one-half.

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A few cases have been reported in which death has been blamed upon the serum, but this is not by any means proven to have been at fault, and considering the immense number of injections which have been made with nothing but favorable results, these cases must be of doubtful nature.

THE PSEUDO-DIPHTHERITIC BACILLUS.

Hoffman, Fränkel, Escherich, Löffler and others have described a bacillus occurring in cases of membranous angina, differing but slightly from the Klebs-Löffler bacillus, some observers even claiming that it cannot be differentiated. They are somewhat shorter and thicker, grow at a temperature of 20–24° C. (68° to 72° F.), forming a mere yellow layer upon agar, and changing the reaction of bouillon less rapidly. They do not grow as well in the presence of oxygen as do the true bacilli. Inoculations into animals sometimes produce local manifestations, but never death.

Hoffman found them frequently in the healthy pharynx; Fränkel, Roux and Yersin think that they are the same bacilli as the Klebs-Löffler, which have in some way lost their virulence. Welch thinks that the name pseudo-diphtheria bacillus should be applied only to bacilli which, though resembling the Klebs-Löffler bacillus, differ from it in being non-virulent, growing more luxuriantly on agar, and the preservation of the alkaline reaction of the bouillon cultures. He holds it to be of a different species than the diphtheria bacillus, and considers it of no diagnostic importance. Paltauf, Sevestre, Baginsky, Martin, Park and Prudden have described cases of membranous disease where the Klebs-Löffler bacillus was absent and only streptococci could be demonstrated. Staphylococci are often found within the false membrane, but they bear no relation to the disease. For clinical purposes, all cases which give bacilli resembling the Klebs-Löffler bacillus in culture and under the microscope should be considered diphtheria.

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THE BRAIN OF THE EMBRYO SOFT-SHELLED TURTLE.

SUSANNA PHELPS GAGE, Ph. B., ITHACA, N Y.

In a paper read before the Microscopical Society last year, upon the "Comparative Morphology of the Brain of the Soft-shelled Turtle (*Amyda mutica*) and the English Sparrow (*Passer domesticus**)," certain questions were raised, which could only be answered by studying the development in the soft-shelled turtle, as: When and how do the characteristic features of the brain in this group of turtles arise? When and how do those features arise which distinguish them from birds?

Professor Eigenmann, who was present, kindly sent me six embryos of *Aspidinectes*, a closely allied genus of the turtle, in different stages of development. Serial sections were made of the heads and mesal views reconstructed. A brief summary of the result obtained is given below. Fuller statement, with illustration, is reserved until more material is studied.

The body of the youngest specimen was 7 mm. long; the form generalised; the face short; the diameter of the eye, one-half the length of the head. A narrow carapace was distinguishable in a specimen, with length of body 11 mm. In the oldest specimen the carapace was 16 x 11 mm., and had the characteristic leathery appearance and markings of the adult. The snout had also the elongated form of the adult. The feet were webbed. The diameter of the eye, though twice as great as in the youngest specimen, was only one-third the length of the head.

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1. As seen from the meson, the most striking difference between the early and late forms of the brain is the general shape. Taking as reference points the center of the geminum, the union of the myel with the oblongata, and the tip of the olfactory lobe, in the youngest embryo the figure formed is an isosceles triangle, in the succeeding stages changing to a flattened triangle by the elongation of the base. The cephalic limb of the triangle increases greatly, while the folding of the caudal part of the brain produces an actual shortening of the caudal limb of the triangle. In the adult *Amyda*, the flattening of the triangle has proceeded to an extreme. The change of form in the brain is apparently greater between the time when the external appearance of the adult is established, as in the oldest embryo, and the true adult condition, than between the oldest and youngest of the above-described embryos. This is due to the fact that after the external adult appearance is complete the cerebrum and the cerebellum both acquire their largest comparative growth.

2. At the constriction occurring in the brain-tube, between the postcommissure and the floor of the cranial flexure, the brain shows the least increase in size, as shown by different measurements upon the meson, of the embryo and adult brain. This stationary condition is probably due to the early maturing of the region.

3. The union of the olfactory lobes across the meson was not found in these turtles until the beginning of the carapace was distinguishable, and did not present the comparative extent and close connection of the adult until the oldest embryo with the adult appearance. That is, as was found with the sparrow, the union across the meson is of late occurrence and secondary importance.

4. Those parts of the cerebrum, apparently connected with olfaction, the hippocampal, progress with equal step with the olfactory lobe, and not until the oldest embryo is the fimbrial edge of the hippocamp and its union across the meson, the fornicommissure, well established. The late appearance of this commissure is consonant with great varia-

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tion in different types, but this study tends to corroborate the opinion now gaining ground, that this commissure in the lower vertebrates is not a callosum.

5. That part of the cerebrum so prominent in the adult, the caudatum, or elevated portion of the striatum, is only found as a rather inconspicuous object in the oldest embryo, but the precommissure, in which fibers from the upper parts of the striatum cross, arises as the carapace begins to form.

6. In the roof of the brain the postcommissure is a well-formed landmark in the earliest of the embryos, while the commissure, bounding the opening of the epiphysis, the supracommissure, shows as a mere trace in the youngest embryo and attains a disproportionate development in the oldest. A similar culmination in growth is seen in the oldest embryo in the associated epiphysis, habenæ and the fiber tract extending from this region to the cerebrum, a fact apparently indicating that in ancestors of this group having comparatively simple brains these parts were of more importance, for in the adult turtle they are overshadowed by the later developing parts.

7. The membranous roof in all embryos is a simple unfolded membrane, clearly continuous with the paraplexuses of the cerebrum. The latter, in the early stages, are simple membranes, which show folds only when the carapace begins to develop, and become quite complex in the oldest embryo. The paraphysis, at the point of union of the diaplexus with the paraplexuses, is a widely open tube in all the stages, and becomes early convoluted.

8. The medicommissure, a feature which is found in mammals and reptiles, but not in birds, arises in this turtle only in the oldest embryo, in this being like mammals, in which it also appears late, and showing that though characteristic, it is of secondary importance.

9. In the infundibular region of the embryo are seen distinct folds and pits, which are nearly obliterated in the adult. A pair of protuberances, dorsad of the hypophysis, occurs in the younger forms, and is represented in the adult by a single

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mesal notch. Dorsad of these, a mesal protuberance, lying between two commissures, is much more prominent in the younger specimens before the commissures are formed. The decision upon homologies of these protrusions of the wall with either the albicans of the higher forms or the hypoaria of fishes must be reserved, for there are details of difference with both.

10. In the turtle, all parts connected with vision are well developed. In the youngest embryo the optic recess is clearly traceable to the eye along the optic nerve, as the remains of the originally open vesicle. This remnant becomes more convoluted, the endymal cells giving an almost glandular appearance, in the stages when the carapace begins to develop. In the oldest embryo this appearance is gone, but the numerous cells of the chiasma in the adult may represent this convoluted vesicular remnant.

11. The optic geminum does not lose the form of a thin-roofed single vesicle until in the oldest embryo a mesal depression occurs, forming the paired geminums, and at the same time an extensive union across the meson by means of the geminal commissure, and a division of the cells into two layers arise. The late formation of this solid roof of the geminums is interesting in connection with the fact that in birds the roof remains a membrane.

12. In the latest embryo the cerebellum is only just beginning its growth as a great mesal feature, though considerably earlier it is apparent as a lateral organ. In the youngest embryo its appearance is like that of the *Amphibia*, having a small mesal portion. With its growth caudad it revolves, so to speak, about a fixed point, carrying the thin membranous wall before it, and thus forms the folded metaplexus of the adult. The oldest embryo shows this admirably.

13. The floor of the oblongata undergoes wonderful changes, from a comparatively thin-walled condition in the youngest embryo, through one in which series of rounded thickenings occur, these in turn becoming united, as the car-

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apace develops, to form the continuous thickened floor of the oldest embryo.

From the above it is seen that partial answers to the questions mentioned are now possible.

(*a.*) The general form of the brain of the soft-shelled turtle wherein it differs markedly from the other described turtles is only acquired after the embryo has the external appearance of the adult, the great relative growth of the cerebrum and cerebellum taking place after that period. (Sec. 1, 2.)

(*b.*) The union of the olfactory lobes across the meson and the large caudal growth of the cerebellum seem to be characteristic of this group of turtles, and it was found that both are of late development. (Sec. 3, 12.)

(*c.*) The broad distinctions between the bird and reptile brain are, that the latter possesses a medicommissure and a solid roof to the geminums; in the soft-shelled turtle both of these features arise in the late embryo.

That is, in the brain not only those features which distinguish the group of turtles, but which most evidently distinguish birds from reptiles, arise in this turtle about the time the external form is characteristic of the genera. The brain, however, lags somewhat behind the body in assuming characteristic features.

Other questions arose as to the appearance of the nidi and their relation to sulci, which cannot yet be answered conclusively.

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NOTES ON TECHNIQUE.

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In many of the modern articles, the methods by which certain pathological structures are demonstrated, if mentioned at all, are frequently so meager in the description of important details as to be practically useless to many workers, unless a certain amount of their time is devoted to experimentation. A person, who has obtained fairly successful results with his older methods, is loath to forsake them, especially if his first few attempts with the new are failures. Each investigator may have certain laboratory conveniences; reagents of the best quality and dyes that have been well tested, all of which will enable him to obtain results much superior to his less fortunate colleague. It is difficult, therefore, to work successfully unless details are carefully attended to, and the reasons for the various steps understood. The methods following have been well tested, and have been attended with uniformly good results, which in some cases, it is believed, would have ended in failure with the older methods.

FIXATION.

The fixation of pathological tissues, with strong alcohol for histological study, is very commonly employed for the double purpose of killing at once any microörganism that may be present and at the same time to preserve the structure of the part. With many tissues this caused a too rapid withdrawal of the contained water or lymph, so that the specimen becomes hard and gives unsatisfactory results when it comes to the cutting process.

Some experiments with different reagents, upon known pathological material, were of service in formulating a mix-

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ture, which obviated the defects of strong alcohol when used alone. This mixture, while quickly killing the bacteria, also preserves most faithfully the histological structure. Various solutions of formalin, including the undiluted, were employed, and gave good results, particularly the presentation of the bacteria, after the usual staining methods. The tissues were more or less swollen by the weaker solutions, in marked contrast to the contraction caused by alcohol. Various combinations of formalin with alcohol were also tried, and that which seemed to be most completely satisfactory for quick penetration and convenience, bacteriologically and histologically, was as follows:

95 per cent. alcohol	100 parts.
Commercial formalin (40 per cent. formic aldehyde) .	10 parts.

Pieces of tissue, $\frac{1}{2}$ centimeter square, are well fixed in from twelve to twenty-four hours, after which it is well to leave for a few hours in 95 per cent. alcohol before clarifying for the paraffin bath. Specimens, transferred directly from the fixing mixture, have been clarified in chloroform or cedar oil, but it requires a longer time.

The addition of the formalin is advantageous, because in a way it brings about a state of equilibrium. The alcohol alone shrinks the tissue, while on the other hand formalin swells it, so that in this respect the one reacts against the other.

ADHESION TO THE SLIDE.

After the infiltration and imbedding of the tissue in paraffin, the question of the treatment of the sections is one of some importance. If they are to be carried through a series of reagents in watch glasses, and not placed upon the slide until they are mounted, the sections must necessarily be rather thick, in order to withstand the manipulation. Very much thinner sections, if adherent to the slide, and consequently supported by it, can be carried through the different steps of the process without injury, and show the structural elements to much better advantage.

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The albumen or collodion adhesive, usually employed for this purpose, however, possesses the disadvantage of taking the aniline colors used in bacteriology, sufficiently to disfigure the preparations. If a clean slide be coated with a thin film of glycerine and then rubbed very nearly dry with a cloth or the hand, and a drop or two of 35 per cent. alcohol be placed upon it, the section, if curled, will tend to flatten itself when placed on the alcohol. If the slide now be placed in a thermostat for a few hours, at a temperature near the melting point of paraffin, the heat will cause any wrinkles or irregularities of the section to disappear; the alcohol slowly evaporates and when the slide is thoroughly dry the albumen molecules of the tissue adhere quite firmly to the slide, as noted by Gaule. After this the slide may be heated gently over a flame until the paraffin begins to melt. If any moisture remains the section will be quite likely to loosen during the latter stages. Thick sections do not adhere so firmly as thin ones. The slides may then be immersed in a jar of turpentine or any solvent of paraffin and carried through the various grades of alcohol to water.

A shorter method, in which there is as firm adhesion of the section to the slide, is to bring the slide in contact with aniline oil for a few minutes after the treatment with the turpentine, absorbing the superfluous turpentine with filter paper. The aniline oil is also removed by means of filter paper. The section is then thoroughly washed in distilled water which removes the oil, and the tissue is then stained and washed in water. If aniline stains are used, a hurried rinsing is sufficient. Drain or absorb the water and again apply the aniline oil. Besides clearing the section the oil tends to remove the aniline stain and care must be exercised in not letting this process go too far. Displace the aniline oil with xylol and mount in balsam. The color ought not to fade if the aniline oil has been thoroughly removed.

With certain stains, or combinations of them, the aniline oil may not succeed in preserving the sharp definition of the color. Under such conditions the section, after staining,

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may be treated directly with absolute alcohol to dehydrate and remove any superfluous stain. Some aniline dyes are not as soluble in absolute alcohol as in the weaker grades. Clear in xylol and mount in balsam.

The use of aniline oil in the treatment of the sections will be recognised as having been recommended by Weigert for bacterial purposes. It likewise gives most excellent results in ordinary histological work and is a saving of time and material.

MOUNTING.

Many valuable specimens are ruined for the want of sufficient precaution in the preparation of the balsam. In its commercial state it contains many volatile principles and traces of acids, which, in the course of time, act upon the specimen and diminishes or entirely removes the color. All this may be lessened, if the balsam be heated sufficiently to drive off the volatile constituents, or more thoroughly obviated if a little potassium carbonate or mild alkali be added to neutralise the acid just before the balsam is heated. When the balsam becomes hard it can be broken into flakes and stored. When wanted for use dissolve in xylol to the desired consistency and filter through absorbent cotton. Specimens stained with the Biondi-Ehrlich mixture (which fades so easily) have at the end of a year shown no signs of losing their pristine clearness.

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SOME METHODS OF HISTOLOGIC TECHNIQUE.*

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In carrying on original work and in obtaining good results in the course in normal histology in the laboratory under our charge, it has been a problem of considerable interest to reduce to the smallest possible amount the time and energy expended in the work of preparing material for teaching a class averaging about sixty students. While it is impossible to lay down any fixed rule in work of this character to be the guide of the student in making preparations for the study of normal histology, we believe that by the employment of simple methods which will meet the largest number of requirements in preparing tissues for examination, and recommending these to the student, we reap the greatest benefit from the necessarily limited amount of time allotted to such a course. While points specially alluded to in this paper are not original entirely, but are the result of long observation of the working laboratory courses of various teachers, I am satisfied that quite a number insure accuracy in work with the least possible expenditure of time and attention.

The student is taught from the beginning the general principles of post-mortem examination, cautioned as how to be selective in material for normal and pathological histology, taught the various methods of staining technique, and preparation of slides for his individual study. To ensure the most accurate results, so far as the relationship of tissues are

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concerned, as well as for their immediate preservation, formalin has been used as a hardening and fixing agent. From the time the selected material starts upon its course of preparation it is designated numerically, and the name of the object or specimen immediately entered in a register, which I shall describe later. A great waste of time and the loss of many valuable specimens result from the placing in the single preservative of specimens which were not previously marked for identification. The method employed now, and which has worked favorably, is to tag each preparation with a specially prepared pure tin tag. The tagging material may be procured in sheet form and of very thin quality, and tags of any size desired by the preparer can be made by simply marking off the dimensions upon the sheet. It is my custom to prepare, say, 400 tags at a time, and number them in serial order by means of small dies and afterward cutting the sheet into strips. The most convenient size I have found to be one inch by three-sixteenths. When cut of this size they take up very little room in the smallest specimen jars, allowing of many preparations to be placed in a single bottle. With a small punch, at the right hand side of the tag, a hole is made, through which a strong, but delicate, linen thread can be attached for the purpose of fastening the tag to the specimen. It is found most convenient to fasten these to the specimen by allowing, say, only 1½ inch of the string to project. If they are made longer, and many preparations are put together in a jar, they are apt to become knotted and considerable delay is caused in separating preparations while passing them from one reagent to another. These tags have several advantages: they are perfectly preserved in any of the common preservative fluids without tarnishing, corroding, or in any way obscuring the number which has been stamped upon them; if dies are not handy, the number or even the name of the preparation can easily be scratched upon the tag with the point of a sharp instrument; they can be used repeatedly if it is a question of economy in the purchase of the material, although it is extremely cheap and if one is

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conducting considerable work it is more satisfactory to keep up a consecutive system of numbering. I prefer the numerical registering of preparations for the following reason: There is no possibility of duplication, and furthermore, with the numbers properly described in the alphabetical register prepared for the purpose, the preparation is known from the beginning to the end by its number, and no possible confusion can result.

The material secured for future microscopic preparation is immediately tagged in the manner before described, and from that time goes forward through the various fixing, hardening, embedding and staining reagents with the tag affixed. If properly attached to the most delicate anatomical preparation they are not likely to get away from it, if ordinary care is used; in dense tissues it is almost impossible, without destroying the tissue, to separate them. It is my custom, when preparing a number of fresh tissues, to give them their respective serial numbers, carrying at the same time through the various reagents until the point of embedding is reached, when a further and special use of the tag presents itself. I formerly embedded all preparations in the more common forms of water bath. So far as the embedding process was concerned, it was as perfect as the method I now use, but it had the disadvantage of always having the paraffin in metal cups, which made it impossible, without exceptional conditions of light, to define one specimen from another. I now employ ordinary glass cylinders, commonly made for staining jars, and place my specimens in the paraffin in a hot-air bath governed in the regular way with a thermostat, After the allotted time for proper infiltration, one can open the door of the bath and readily see any preparation through the clear melted paraffin in the glass cylinder. As it is of importance just at this stage of preparing, I should have stated that it is particularly necessary to attach the tag to the specimen in such a way as to indicate that the preparation should be placed in the imbedding block in a particular position. It takes but a moment to do this at the time of cutting

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out material, and saves time, which is of great importance, at the actual moment of embedding, when, if the process is delayed too long, the saturated specimen stiffens and the surrounding paraffin in the mold is not of the same density. The special advantage of the tagging system at this point, that is, in transferring the specimen from the bath to the cutting block, is that you can seize the tag with your forceps, withdraw the specimen from the melted paraffin and instantly transfer it into the mold which is to receive it, and which has previously been filled with melted paraffin. For these molds I have used for a number of years small sections of lead pipe, which I keep in stock of four or five different diameters, also of several different sizes in lengths from $\frac{3}{4}$ inch to 2 inches. The advantages of molds of this kind are that they are sufficiently thick to be firm on the base, when touched with a little melted paraffin and placed upon an embedding slab they are firmly seated and prevent the melted paraffin from running out at the edge, and furthermore, when the block becomes hard with the enclosed preparation, the specimen is without any trouble or unnecessary pressure removed from the mold. The specimen is transferred from the bath to the embedding mold, and when placed in the desired position for cutting, the metal tag is allowed to drop over the edge of the mold. As will be readily seen, if you remove either a few or a large number of embedded blocks from these molds, the firmly-attached tag serves to identify the preparation at any time. As there is always an excess of paraffin about a piece of tissue, I immediately trim away until the block is in a suitable shape for the jaw of the microtome, taking care not to cut the attached string, and after it is properly prepared I gently heat the bottom of the paraffin and press my metal tag directly onto it. This keeps the number conveniently attached to the block for future use.

Another point of great convenience I have found to be in the employment of carton boxes, of the character and size accompanying this paper. It was formerly my custom, in

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the care of a large number of preparations, which were accumulated from years of hospital and laboratory work, to wrap the paraffin blocks in special paper wrappers and number and label these; they were then kept in compartments accommodating fifty each, in numerical order. As few preparations were of uniform size, the collection of blocks for ready reference in this way was far from satisfactory. As soon as a block has been prepared for the microtome it is immediately placed in one of these carton boxes, which is numbered to correspond with the preparation; these boxes being uniform in size, and adapted to the largest sized preparations commonly used, are most conveniently kept in a cabinet containing shallow drawers which will hold 200 boxes each, one layer deep. The cabinet I use consists of six such drawers, will hold 1,200 blocks arranged in this way, all in numerical order, and turning from the index to the cabinet, any desired block can be secured at once, and of course is readily returned to its place.

Concerning the matter of indexing one's material in a large laboratory, I find the following plan to work to my entire satisfaction, having had it in practice for about five years, and it has met all my requirements. I divide my material, as the work in my laboratory has been duly divided in the normal and pathological instruction, into these two respective classes—Normal and Pathological. Taking several of my most comprehensive works treating of each of these subjects, I analysed the indices and prepared an index of my own, which was the result of getting together the essential points contained in these text-books. Using an index book, I then placed in it, in strict dictionary order, the essential subjects which I expected to collect in normal histology and in pathology. In my register proper, contained in the same book, I devote one-half of it to the normal subject and the other half to the pathological. The material that I had at that time, which was not of small amount, I then entered, allowing a suitable division of space for the title, date, and description, and any memoranda relating to it in its proper place on the

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page of my register, where it bears a corresponding number of the block. Under the subject, then, to which this particular preparation belongs, say angioma, for example, I refer to it in my already prepared index simply by number. The index, on the whole, was a very complete one in the beginning for the purpose for which it was intended, and I have added very few titles in the long time it has been in use. It shows at a glance about how much material I have of any subject, refers me to the blocks instantly by number, and shows me in what material or in what subject I am scant, or in which I have nothing at all; in other words, it is a good index of the desiderata, as well as what you have on hand. In my register, as I receive material, I enter the title of it in the order it is received, whether it be normal or pathological. In my index I separate the normal from the pathological, although contained in the same alphabet and upon the same page. I index the normal subjects in black ink and the pathological in red. With an index of titles of considerable size, as this one is, I believe this method reduces to the least possible space the large number of subjects, and still allows ample room for the entering of a great many reference numbers; this serves the double purpose of an index by referring to the proper location of that particular material in the register, as well as to the block, and its location in the cabinet.

Just another point regarding matter of technique, but one which may prove useful to some member of the Society, as it has proved to me. In the last dozen years I have followed through all the various stages of fixing preparations to the slide for staining purposes, and have tried with more or less success about everything that has been recommended for this particular purpose. During the last few years I have used the common gelatin mixture which is recommended by Dr. Piersol and Dr. Gray, but found that in certain methods, while it had many advantages, it had its disadvantages also. The most trouble was experienced in staining with the hematoxylin stain and contre stain. I found when it was

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used according to the formula first recommended, namely about half per cent., that it was not uncommon for the extremely delicate gelatin film to take up a small amount of the stain, especially about the border of the preparation. I used it even as dilute as one-tenth per cent., and yet, in some cases, with the same troublesome result. Every effort and much time was spent in freeing the slide from the excess of the gelatin mixture, but, nevertheless, there was always a small amount of it perceptible.

I am indebted to Dr. Walter Reed, U. S. A., for the suggestion of the method which I now employ, and have used with perfect success. Knowing that he was using similar methods of staining, I asked his experience as regards a fixative. He informed me that he had, in some cases, similar trouble, and told me that he was using only distilled water for the purpose of fixing sections on the slide. I followed his suggestion, and since that time, now nine months, it has been extremely rare for the preparation to become detached from the slide. I will say that, as an illustration, I cut and fastened to the slide, by this method (simply placing the section upon the distilled water), 1,640 individual sections. These were afterwards carried through the usual process of staining, cleaning, etc., and without the loss of a single section. While the method is in the main commonly understood, it may not be out of place to say, in a few words, how time and labor is saved in this part of the work. It is commonly my custom to prepare twenty-four or more slides at a time of a particular object; I place these in a tray holding two dozen, which is commonly employed with the ordinary drying bath, the slides depending upon the size of the section, are covered with distilled water, and the sections cut and placed upon the water. The entire set is then placed in my drying oven sufficiently long, and at a proper temperature, to smooth the sections entirely, taking care that the temperature never reaches the melting point of the paraffin. The tray of slides is then removed, each section accurately centered in the desired position on the slide, the excess of water allowed

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to run off, and the slide placed upon end in a rack for drying. The racks that I employ, while not original in any sense, are well adapted to this special part of the work, and I submit one for examination. They are made to hold seventy-two slides, a very convenient size, and of a material which will not warp and allow of an irregular arrangement of the slides.

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HISTOLOGY AND METHODS OF INSTRUCTION.

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In the preface to the first edition of his *Handbook of Human Histology*, Kölliker made this significant remark: "Medicine has reached a point at which microscopical anatomy seems as necessary for a foundation to it as does the gross anatomy of the organs and system; and when a profound study of physiology and pathology is impossible without an exact knowledge of the finest structural details." If this was in the main correct in 1852, when Kölliker first wrote it, how much more is it so at the present day when not only medicine, but the great science of biology is taking such a prominent position in the minds of men. Indeed, in its broad aspect medicine is but one of the details of biology, and pathology is biological activity perverted by abnormal influences and environment; and since the time when Virchow's cellular pathology appeared, it has been known that the real seat of this perverted activity resides in the microscopic elements or cells which compose the different organs and tissues. Likewise is it known with the greatest certainty that all normal activity goes on in the microscopic elements making up the tissues; and finally the germs of a new generation, the bearers of heredity by which the past reappears in the future, are likewise, in most cases, microscopic elements. In a word, without the microscope, knowledge would be turned back a century and the certainty concerning many things in biology today would give place to the baseless speculations of the dark ages.

All teachers of histology have, of course, the same general object in view, viz.: to give their pupils a knowledge of the

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microscopic structure of the body. Naturally, and of necessity the way in which different teachers go to work to give their pupils this knowledge will depend on the teacher's view as to the special end to be attained by the study, and secondly on the facilities he has at his disposal. The views expressed in this paper may not accord with those of teachers, in whom experience and special surroundings have given rise to fixed convictions, but it is hoped that some of the younger teachers may get suggestions from it that will aid them in making the most of their surroundings and facilities; it is hoped also that the subject of histology will be seen by them to be vitally important for an understanding of physiology, morphology and pathology. It is hoped also that the end of histology will not seem to any to be reached when an organ or tissue has been fixed, hardened, cut with an expensive microtome, stained in brilliant colors and finally embalmed in Canada balsam. It is hoped rather that all of this labor and pains may be seen to be only to help one see the physiologic, morphologic or pathologic processes and relations exhibited by the tissue more clearly. If the microscopic preparations have no such meaning to the student then they are no better than so many Chinese puzzles.

It seems to the writer that the first step in histology is a thorough study of the chief instrument used, the microscope. The microscope is to aid the eye in seeing what is invisible or not satisfactorily visible without it, and unless one knows something of the methods of making this helper to vision a real helper, much time will be wasted. This is especially true of the better forms of instruments. One can use with some satisfaction a simple magnifier without instruction or much study, but a good modern, compound microscope to be of much use must be well understood; one must know its possibilities and limitations. It seems to the writer that time is really saved for histology by devoting a few weeks to the microscope itself, and to the methods of micrometry, drawing, the use of the micro-polariscope, the micro-spectroscope and other accessories. Otherwise one must learn

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these things when he is trying to make use of them in solving some problem in actual work.

It may naturally be asked what kind of a microscope is necessary for the pursuit of modern histology? While a great deal of excellent work may be done with comparatively inexpensive apparatus, costing from $25 to $50 and magnifying from 25 to 500 diameters, one cannot follow out the finer details in histology and pathology with such an outfit, and in some parts of pathology, where bacteria are involved, one would be practically helpless. Some such outfit as the following seems necessary: Dry objectives of 50 mm. (2 in.), 16 mm. ($\frac{2}{3}$ or $\frac{3}{4}$ in.), and 3 mm. ($\frac{1}{8}$ in.), and a homogeneous immersion of 2 mm. or $1\frac{1}{2}$ mm. (1-12 or 1-16 in.) There must be some form of substage condenser. This, like the objectives, will serve one in proportion to its excellence. The stand of the microscope should have a coarse and fine adjustment for focusing, the pillar should be flexible, so that it may be used in either the vertical or inclined positions, and the substage should have a rack and pinion adjustment for the substage condenser, and an arrangement for centering. Fortunately such an outfit can be had at the present day for less than $100, if supplied with ordinary achromatic objectives; but the cost is much greater if the best achromatic or apochromatic objectives are obtained. It is of the greatest advantage also to have a mechanical stage of some sort. The removable mechanical stages after the Tolles-Mayall pattern are inexpensive and most satisfactory.

For laboratory work there are two methods, the one allowing students to come at their convenience and accomplish as much work as they can or wish to. The other plan is to give a medium amount of work, which must be accomplished in a given time. The students are required also to come in regular sections. The last way seems to the writer the best. Experience has shown that regular sections, in which the teacher devotes his whole time to the laboratory, yield better results. There is a kind of momentum gained in this way that overcomes the inertia of the less energetic,

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and for those that get through with the small amount of work that must be assigned for a lesson there is abundant opportunity to consult monographs and go more deeply into the subject than is required of the average student. To conduct a class in this way, however, necessitates abundant, well-lighted space, plenty of tables and microscopes, and other laboratory facilities. It can be readily seen that laboratory work in histology carried on in this way requires an expensive plant. If the subject is to be taught at all, this is the only economical way, however. To keep a laboratory open all day and every day, the teacher being on duty all the time, is wasteful and the results unsatisfactory ; as unsatisfactory and uneconomical as it would be to divide a Greek class of twenty up into five to ten sections for recitation. The last section would hardly gain much inspiration from the teacher, and such a teacher would not be likely to add much to comparative philology or anything else.

In the actual instruction it is believed that there should be a combination of lectures and laboratory work. The lectures serve to give the students broad and general ideas and the relations of the subjects to each other ; that is, they give the fundamental facts, principles and relations, which are the result of the investigations of the best workers. The best books and monographs are referred to and shown, and put at the students' disposal. This is done because it is believed that every one should take advantage of the gain made by his predecessors and not try to start at the beginning. Life is too short for that, and progress would almost or quite cease if the gain made by our predecessors could not be made use of. From a long observation it is believed that the student who has the power to make independent investigations should have these helps, so that he may recognise the attainments of others and start from their vantage ground to explore new fields. For the student who has not the power for original investigation this is the only way to help him. He cannot go where there is no path.

In the second place there should be abundant opportunity

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for laboratory work where the student is brought into direct contact with the truths of nature in nature herself, and if he is an honest man he must work very hard to make out these truths, no matter how much help he has been given by lectures and books.

In the laboratory work each student should learn and practice all the principal methods. A preparation made by the student himself from getting the tissue until it is mounted and labeled means something to him ; it is connected in a very definite way with the organ or part in the animal. He also gains skill in manipulation, and without skill in manipulation no real progress can be made in any science. Exact notes, with dates and drawings, are necessary to avoid vagueness and to prevent the student from deceiving himself in the belief that he has gained certain knowledge when he has not. These notes and drawings, and the students' specimens, duly labeled and catalogued, should be most conscientiously scrutinised by the teacher. They give him an opportunity that nothing else can to help the student by correcting erroneous conclusions and by aiding him in gaining skill in manipulation. It may well be asked, however, if it is possible to get a class through the tissues and organs of the animal body by having each student perform all the operations for himself. It is admitted that the time necessary would be too long, and for most of the students much time would be unnecessarily used in mere mechanical operations. The plan advocated is to have each student learn all the fundamental processes in modern histology, and learn them by repeated operations, but the loss of time by mere repetition after the processes have been mastered may be avoided without injury by furnishing most of the preparations either already cut or imbedded ready for cutting. It is believed that every preparation, with rare exceptions, should be in part at least, the work of the student. If then for these partly prepared preparations full data are given concerning the methods used the student will have no trouble in making the proper connection mentioned above when he performed

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all the work himself. It is believed that the ground can be covered in this way and it is known from experience and observation that the intellectual independence gained by the personal work of each student will repay all trouble on the part of the teacher—for it is more trouble to guide the student than for the teacher to do the work himself. The student will gain also the power to use the work of others, and to judge it at its true value as he could in no other way.

In the actual work carried on by the writer, lectures are given to the entire class, and, then, for the laboratory work sections of about fifteen are taken for not less than two hours at a time. If a period of less time were given, so much of it would be used in getting ready to work and in clearing up that not enough actual, productive work could be done to repay the effort. Each student is given the use of a locker ; each one prepares nearly all of the reagents used by him, and each one learns the methods of isolation, of sectioning by the collodion and by the paraffin method, both with simple and inexpensive and by the best modern apparatus ; and all have opportunity to see the method of making frozen sections, so largely used in diagnosis in pathological work. There is a large cabinet of specimens illustrating microscopy, histology and embryology, made and labeled and catalogued with all possible care, to serve as models for the students and for reference. The cabinet has been found very valuable for stimulating independent work. If one sees only figures of microscopic objects he may feel that to make actual specimens which shall show the objects with equal clearness would be impossible for a student, but if such specimens are at his disposal he is stimulated and encouraged to prepare similar ones for himself. He soon learns also, in studying actual specimens, that many of the figures in the books are composites,—made by combining the best features of several preparations.

For convenience, the animal body is divided into the following groups of tissues and organs. The arrangement is

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more or less logical also on embryologic, physiologic and morphologic grounds:

1. Epithelia, including endothelia.
2. Connective and supporting tissue (Areolar tissue, tendon, ligament, bone, cartilage, etc.).
3. The muscular system.
4. Blood and lymph, *i. e.*, the fluids of the body and their corpuscles.
5. The blood and lymph vascular system.
6. The digestive system.
7. The respiratory system.
8. The genito-urinary system.
9. The skin and its appendages.
10. The nervous system and the organs of sense.

In teaching, the following guiding principles have been followed:

1. It has always seemed to the writer that one of the most important steps in the knowledge of the structure of the tissues and organs is a thorough knowledge of the gross anatomy. The histologist must, first of all, be a thorough naked-eye anatomist. He must also be a physiologist, and he will naturally become an embryologist, for without the knowledge that embryology gives, the adult structure is frequently unintelligible, and without physiology, structures are, in many cases, meaningless. The wise histologist is then a physiologist, an embryologist and an anatomist. From the naked-eye appearances he passes as necessity requires, from the contemplation of organs and tissues, first to a low power and then for the finer and finest structural details to the highest powers available. But he never loses sight of the fact that the details alone are far less intelligible than when they are correlated with the organ or tissue to which they belong.

2. It seems so natural and logical in teaching the fundamental facts concerning the morphology and structure of the body to refer to the mode of development, that for several

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years the students have not only been taught in lectures from the embryological standpoint, but each student in the beginning has put into his hands, in the laboratory, preparations of the ovarian ovum to represent not only a typical cell, but the fundamental fact that the complex body of the largest animal is derived from the ovum. Then preparations of the blastula with a single layer, representing in a general way a simple epithelium, are studied, and then the blastula with a wall several cells thick, representing in general a stratified epithelium. Other preparations are studied, showing clearly the mode of formation of the axon or notochord from the entoderm, and of the neuron or central nervous axis from the ectoderm. After studying these preparations it means something to the student when he reads or hears in lectures that a given tissue or organ is derived from one or the other of the germ layers.*

3. Each tissue is studied fresh, so that correct notions may be gained of the natural appearance of the organs and tissues and their structural elements unaffected by reagents.

4. Every organ and tissue is studied alive, so far as possible, in order that the function and the structure that performs the function may be seen at the same time and the two properly associated. Students who see only prepared specimens can hardly avoid gaining the impression that the gorgeous red, blue and purple colors belong to the natural tissues, and would be so found in dissecting an animal. Indeed the histologist who studies his subject profoundly looks upon the adjuncts of stain, etc., as necessary evils at best, and he never feels quite sure that the appearances seen in these much-stained and manipulated specimens are true expressions of nature, or whether they are structures of his own creation (artifacts), until he has seen the appearances in the living substance, where the pitfalls of color and Canada

* The preparations used in my laboratory are the small ovarian ova found in the ovary of a young *Amblystoma*, or those left after spawning. All sizes are seen, giving also a hint that the different sizes mean the different crops of eggs, so to speak, that will reach maturity. The segmenting ova of *Amblystoma* are admirable for showing the blastula, and the formation of notochord and nervous system.

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balsam have no place. (See the preface to *Foster and Langley's Practical Physiology.*)

5. All glands should be studied in various phases of their activity and repose, so that the structural features present in each phase may be associated with the functional condition. In a word, it is greatly to the advantage of the student if the histology he studies is truly "Physiological Histology."

6. The student will gain a truer insight into the structure of the body if he understands at the beginning that every organ and every tissue as it is found in the body is really a complex; that is, it is composed of several tissues and of ground substance. For example, muscle is composed not only of the characteristic structural elements, the muscle fibers or cells, but mingled with these are connective tissue and blood vessels, and nerves are abundant. Even in epithelium the cells are not the whole of the tissue, for there is always present the cell cement uniting the cells. In connective tissue, the characteristic elements or cells, so prominent in this tissue in embryonic life, are so far pushed into the back-ground by the intercellular or ground substance, that the tissue is actually characterised, not by the cells, but by the ground substance. Thus we speak of cartilage, ligament and bone and the other members of the connective tissue group, having in mind almost altogether the intercellular substance, and not the cellular elements.

7. Of necessity, as well as preferably, every general course in histology must be a course in *comparative histology*, as structural details are not all shown with equal clearness in any one form and not obtainable at all, or only with difficulty in some. For example, hair is not found below the mammals, and the fibrin network in the blood and lymph is far more satisfactory in man and the other mammals than in *Amphibia* and fishes, while nucleated red blood corpuscles are found with difficulty in mammals, while they are normal in non-mammals. As the course is then to be really one in *comparative histology*, the fact should be distinctly expressed, and the student not left to infer that a structural detail seen

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in one animal would be found exactly similar in all others. On the other hand, it should be most emphatically brought out that *while there is unity in type there is much diversity in detail.* This can be demonstrated by each student in comparing the striated muscle of mammals and *Amphibia ;* or to take nearly related forms, the *ligamentum nuchæ* of the ox and other grazing forms is almost purely elastic tissue, while in the cat and man it is largely white fibrous tissue, and far less prominent. This point has been insisted upon, because if any one looks through the pages of any work on histology, even though "human histology" may be printed on the title page, he will find it really a comparative histology, with the comparisons left out. That is, there will be figures of structures from widely differing animals to illustrate the structure of the different tissues, and frequently even the accompanying legend or explanation gives no hint that the tissue figured is not from man. Naturally the student concludes that the tissues are exactly alike in all animals. If on the other hand homologous parts from different animals are carefully compared many of them will show marked differences in detail, although the type of structure is unmistakable.

8. If it is necessary to keep in mind the differences in anatomic details in different animals, so is it equally important to know and to learn to demonstrate differences in structural detail of the same tissue or organ in the same animal in different phases of activity, in vigorous youth and in senile decay. Indeed, the differences in structural appearance of the pancreas, for example, before and after secretion, is as great as the apparent structural differences in quite widely differing forms. It is, therefore, necessary for a complete understanding of structural appearances to keep physiology constantly in mind ; and as so few animals are in perfect health, possible pathologic variations from the normal appearance must be looked out for, otherwise one might in a limited number of observations decide that merely temporary or even abnormal structural appearances were characteristic of the animal under investigation.

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The above statements, while they apply to the study of histology in general, have special reference in the main to elementary courses, where the students are introduced to the subject and are naturally imbibing the spirit of the study.

The course outlined above would require considerable time. It could not be satisfactorily gone over in less than one college year, in a course consisting of two lectures per week and three laboratory periods of two and one-half hours each.

For research in this, as in any other subject, there must be great liberty as well as good facilities for work and experimentation. Mistakes will be made and time apparently wasted; but the mistakes and the apparent waste of time are a part of the "dead work" that must be done by all those who aspire to perform truly advanced work and to add to the sum of human knowledge.

Besides the numerous addresses and special papers that have appeared the student and teacher will find the six books named below especially helpful and inspiring:

An American Text-Book of Physiology. Edited by Wm. H. Howell, of Johns Hopkins. The writers besides the editor are: H. P. Bowditch, J. G. Curtis, H. H. Donaldson, F. S. Lee, W. P. Lombard, G. Lusk, W. T. Porter, E. T. Reichert and H. Sewall. Philadelphia. 1896.

Bernard, Claude, Cours de physiologie générale du Muséum d'histoire Naturelle. Leçons sur les phénom nes de la vie communs aux animaux et aux végétaux, two vols. Paris. 1878-1879.

Foster, M.—A Text-Book of Physiology (1877 to 1896). London and New York. The sixth edition contains much histology. All the editions correlate structure and function in an admirable way.

Metchnikoff, Elias—Lectures on the comparative pathology of inflammation, delivered at the Pasteur Institute in 1891. Translated from the French by F. A. and E. H. Starling, London. 1893.

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Hertwig, O.—The Cell; Outlines of General Anatomy and Physiology. Translated and edited by M. and H. J. Campbell. London and New York. 1895.

Wilson, E. B.—The Cell in Development and Inheritance. Columbia University. Series IV. New York and London. 1896.

For an excellent article on The Importance of Technical Instruction in our Medical College Laboratories, see Dr. A. P. Ohlmacher, *New York Medical Record*, Vol. LXIII., March 21, 1896, p. 374.

For a view that all microscopical and bacteriological knowledge is of no assistance in either medicine or biology, see Dr. Charles G. Kuhlman, in the *St. Louis Medical and Surgical Journal*, Vol. LXX. April, 1896, p. 201.

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WHAT IS THE BEST METHOD OF TEACHING MICROSCOPICAL SCIENCE IN MEDICAL SCHOOLS?

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During the last decade, few questions in medical sociology have attracted greater attention than medical education. The requirements of our colleges not being equal with those of other countries, nor with other departments of education in this country, it was but natural that the profession, as a whole, the medical press and societies should join in a demand for needed reforms. My apology is due to you for bringing this subject before you in a necessarily imperfect way; but I hope for a discussion of ideas, and to learn other ways and means, rather than to give much of value.

By microscopical science, the scope of this paper includes those subjects taught in medical colleges wherein the microscope plays an important part. We shall consider:

(*a*). How much time should we devote to these subjects, out of a four years' course of nine months each, including holidays and examinations?

(*b*). In what years can the subjects most profitably be placed?

(*c*). By what methods should each of these subjects be presented to the students?

1. Lectures and recitations.
2. Laboratory work.

(*d*). What are the best modes of testing or giving credit for work done in each branch?

(*a*). In arranging a medical curriculum, it is a matter of great difficulty to divide the time fairly among the several subjects, giving each its due amount in proportion to its

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importance. In far too many medical schools, a proper *balance* is not preserved, one subject being allowed to usurp time really belonging to another, to the great detriment of the student. This is especially prone to occur where there is a considerable difference in the teaching power of the professors of the respective chairs.

Not long ago the committee of the Association of American Medical Colleges, devoted to bacteriology twenty-five lecture hours and 150 laboratory hours, a total of 175 hours, while in pathology, fifty hours of lecture attendance and 100 hours of laboratory work were deemed sufficient—less by twenty-five hours than the time required for one of its subdivisions! Some colleges prefer to give only two hours a day to laboratory work and one to lecture. In such work as bacteriology this seems to me unfortunate, especially when the hours are arranged with an interval either too long or too short. Many students lose the value of their cultures through long waiting, or the growths have time to become contaminated.

(*b*). As regards the placing of the several subjects much difference of opinion exists. Histology with embryology usually comes in the first year. It may be questioned whether it would not be much better to put embryology in the first half of the second year, when some knowledge of osteology, elementary anatomy, physiology and histology has been taught. Many colleges place bacteriology in the first year. This is to be strongly condemned, I think, because bacteriology requires a great deal of dexterity in handling tools and instruments, including oil-immersion lenses, and also a fair knowledge of physiologic and organic chemistry, and some acquaintance with diseases. I am here referring to a complete course, and not to the mere elementary outlines and details so often taught. The proper place seems to me to be in the second half of the second year, after the chemical course is finished, when we have had some elementary general pathology, when the question of saprophytic bacterial poisons can be better understood. The formation

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of sepsin and its resultant irritative action on the tissues plays an important part in the study of inflammation; and the infective circulatory diseases certainly can be better appreciated after an acquaintance with bacteriology. The same is true of the infective granulomata. What can we intelligently teach of tuberculosis or leprosy if we know nothing of the bacilli occurring in the same, and their effects? Without any doubt, bacteriology should go hand in hand with pathology, or closely following it, and both should have been preceded by some practical laboratory work in histology and elementary biology. Indeed, we have no business to teach pathology to students who have not completed theoretical and practical histology, as is now so often done, especially in colleges that accept the B. A. degree as an equivalent of one year's work. Only a science, and *never* a classic degree should be accepted as such equivalent, and it should include Latin or Greek, German and French as well as technical work, which I believe at the present time it does in most universities. No student can study pathology profitably without first knowing his histology, and such an attempt should be forbidden by the Faculty of the school. Unfortunately the rule too often stands "each man for himself," each professor knowing and caring but little of any chair but his own, and the student certainly not the wisest judge of the order of his studies. I enter an emphatic protest against the faulty grading of students. One who is allowed to begin work in a course for which he is not prepared is a hindrance to the demonstrator, a detriment to the whole class, and besides, he is seriously wronged himself, since he cannot understand what he is trying to learn. The only resource for him is a special tutor.

How then shall we arrange the teaching to obtain the necessary practical results for a medical student in a limited time, with limited apparatus and demonstrators?

If the first year is divided into first and second semesters, let the class begin anatomy and general biology, as outlined in Parker's or Campbell's elementary biology, or in Huxley

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and Martin's. Then follow with the dissection of a cat or dog, using Howell's Dissection Manual. The frog is rather small for beginners, but those who prefer it can work easily from Marshall's little book, or McAlpine's Zoological Atlas. Martin and Moale, Vol. III., is a good guide to the dissection of a rat. The particular animal and text-book selected is of minor importance. Any one of them, carefully worked through, will fix the main anatomical and osteological facts, and give the requisite manual dexterity for later work on the human body. Nothing is more absurd than to place raw medical recruits beside a cadaver, after reciting a few lessons from a book, and expect them to dissect half a human subject in six or eight weeks, working two hours a day. Much better, more intelligent work can be done after the general preliminary training advised above. Elementary chemistry can well be taught at the same time. Indeed, the repetition of terms in the different classes, the explanations given and positions indicated, all amount to a practical translation of the Latin terms in osteology, myology and so forth, and serve to fix in the Freshman's mind what otherwise would be lost.

Embryology is best done in the spring months, when eggs can be easily obtained. In the meantime, histology is occupying some six hours a week, four in laboratory and two in lectures and recitations. Here we are increasing our dexterity and learning the application of anatomy and physiology, and their intimate relations with each other and with chemistry. In the histological laboratory, what shall we teach the student? First, an intelligent *use of the microscope*, its construction and optics. Then methods of hardening, cutting and staining sections, each member of the class, in turn, being instructed to cut sections with the freezing microtome and bottle and label for the class use, so that there are always plenty on hand for testing any desired staining method. And finally, a systematic study of the histologic structure of the tissues and organs of the human body.

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In bacteriology, no single text-book fulfills all requirements. Abbott is very useful, but personally, I think, for a first book, Kanthack and Drysdale's little work is most excellent ; for if a student be given a culture, all details are practically laid out for him, to the great economy of the demonstrator's time, the book being on the plan of the old standard and excellent form in Huxley and Martin's biology.

The student in bacteriology must be taught to make clean, well-stained preparations of some organism, with exactness, so that every *bacterium* is sharply seen when examined. The poor, hazy, half-stained and often milky smears allowed are too commonly found in students' hands and reflect no credit on teacher or pupils. In addition to making neat, accurate mounts, the preparation of media and making inoculations show the student *how to work*—which is far the most important—namely, in a clean, orderly, concise manner. Elaborate methods and appliances should be avoided as far as possible, so that the student can soon learn what is essential and how he can best obtain or make things for himself, after he goes out from the laboratory. Assign to one student a given subject, and require a complete examination and report. Teach him how to proceed to make an original investigation, what are the necessary steps to follow, how to isolate and obtain his own pure cultures from raw material. When he can do these accurately, he can safely be said to know the main processes and principles, at least, in making a bacterial investigation, no matter where he is situated. And this is precisely wherein, I think, our graduates of medicine are lacking. Few of them are able, after leaving college, to proceed to make a complete investigation, on their own resources. Their knowledge of classification, experimental inoculation and subsequent recovery are too vague to be trusted, even by themselves. It is not right to urge that all laboratory work should be out of the way by the end of the second year, as many colleges do, by requiring junior and senior students to spend all their spare time at clinics, forbidding them to take laboratory courses during the third and

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fourth years. It is a fact that students are very prone to get their earlier work hidden away in deep recesses, and to forget how to do things they formerly did well; and it is easy to understand how a student who "finished" his general pathology and bacteriology in the second year may not be able to apply them to his practical medicine and surgery.

General pathology should commence in the second year, and from the first it should be the aim of the instructor to show the deviations from the normal by comparison, and by demonstration of actual specimens, under supervision of the lecturer, who has arranged typical places in the field, and has illustrated them by the lantern. In this course, celloidin and paraffin cutting can be given, as well as special modes of staining, particularly each of the special reactions, as amyloid, and the like. Double staining can here be practised to advantage, and the specimens can be then studied and drawn. Personally I think drawing is best left out after the biologic and histologic courses, because it takes a great deal of time which the student can ill afford to spare. Do not infer that I wish to depreciate this important subject. In science courses, it should certainly hold a strong place. But in medical schools the time is too short, and many of the students have to do outside work to pay their way through college. For the majority of our classes, utility alone is to be considered.

Of far greater value than the ability to draw well, is the practical application of the knowledge required. Pathology, in its truest and fullest sense is to be defined as the theory and science of medicine, to the understanding of which normal structure and function are necessary. True, it is impossible to teach all medical science from the chair of pathology, but the pathologist should maintain clearly the relation which disease bears to the normal bodily condition. He must detail, as completely as the state of science permits, the altered modes of function of diseased organs, the external manifestations of these altered functions, and their remote and direct effects upon the economy. The habit of teaching

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pathology as a bare mass of facts with no relative bearings upon physiology, clinical medicine and therapeutics or surgery is much to be deplored. It is no wonder students so taught regard mounts as an everlasting bugbear and nuisance.

The study of medicine comprises three well-known groups of subjects:

(*a*). Preliminary studies—anatomy, physiology and chemistry.

(*b*). Developmental—those branches which teach the causes of disease, and the altered functions met in disease—that is, pathology.

(*c*). The application of these studies to the recognition and treatment of disease—that is practice and surgery. This shows us that pathology is an intermediate subject between biology and practice. As certainly as the gun-shot wound, the tubercular joint, the fractured bone, or the cancer of the liver take their places in the pathological museum, so should the altered function be regarded as a pathologic study. The man who recalls from his book that renal or cardiac disease may often present, as a symptom, dropsy, is far more liable to miss entirely the vital point in his examination for a diagnosis than the man who has been taught to regard dropsy merely as a manifestation of a circulatory fault, and who has been trained to seek carefully for the arterial disease, or for the cause of venous or lymphatic insufficiency along the courses of these currents whenever examination is possible. The failure to acquire habits of pathologic reasoning, of comparing the diseased structure to the normal, the diseased function with the normal function, of seeking for etiologic influences within as well as without the body is often never overcome. The sneering remark that the pathologist only makes a post mortem diagnosis, has too much truth to be overlooked. This overweening influence of the importance of pathologic anatomy, the confused pathologic histology and microscopy, as applied to medicine, has been the cause of the great impetus given the anatomic school of

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pathology by the great Virchow and through it our advance in this line of work has come. There is a pathology beyond this, however, and for the general practitioner our schools ought to make every effort not to turn out specialists, but careful, conscientious, scientifically educated, practical physicians, with the further privilege of study for higher or future specialistic work if they so desire. For convenience let us divide pathology into three well marked schools:

I. Anatomic pathology, of which the learned Virchow, Ziegler, Klebs and Gross are the best exponents.

II. An etiologic school, at whose head stands Koch and Sternberg, the bacteriologists.

III. A physiologic is, but unfortunately, the least developed, but the most applicable without any doubt to the wants of the busy practitioner of medicine, for example Von Jaksch and Vierord and Osler.

The first was created by the doctrines of cellular pathology of Virchow and has since advanced by the increased facilities of observation.

The second was made possible by the perfection of the microscope and its appliances through the energy of Zeiss, Tolles and by the apparent exhaustion of results of our anatomic study of diseased tissues. Observations naturally drifted towards examination in the existence and nature of parasitic microörganisms and the brilliant successes of this second school, the etiologic, have caused bacteriology to almost outgrow its relative importance in the study and teaching of pathology and almost, if not quite, occupy as high or a higher position. While the third, the pathologic physiologic, has been limited and failing to attract notice by experimental work and popularity, because of the difficulty of demonstration and proof of its principles and details, has had little or no growth and so far has received from teachers of pathology not half the attention its practical value demands. It is this division which makes a man an intelligent practitioner and the ignorance of which makes of him the routine follower of other men's methods.

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No one science should be taught to the exclusion of the other and especially is this so of bacteriology. It is proper to teach the nature of microörganisms which are causative, say of ulcerative endocarditis, that we instruct as to the gross and microscopic changes eventually made in the affected valve when the acute stage is over, but at least remind him of how these changes make known their presence, why they must finally end in cardiac hypertrophy and degeneration, and further how these subsequent alterations indicate their presence, and why such abnormalities in the heart must result in vascular changes, in lungs, liver, spleen, kidneys and so forth, and how he may estimate somewhat the rate of progression and what termination he may expect. Just as we know therapeutics must always belong to the order of applied sciences, and will bear the same relation to pathology in an extended sense as does engineering to mathematics, therefore, as engineering is applied mathematics so therapeutics will be applied physiology and pathology.

One of the most important obstacles to real progress in scientific development and accuracy, is the want of perseverance on carefully devised plans for insuring completeness of investigation before announcing results. Much of our scientific and experimental work is done in fragments and necessarily results in the development of isolated facts and partial views, filling the pages of literature with everchanging and often contradictory inductions. Another obstacle, and a serious one, lies in the frequent formulation of inductions, or more properly assumptions for practical guidance, founded on an inadequate number of facts, and sometimes on mere analogies. Still a third obstacle is the constant tendency to concentration of all attention on the results of any new line of investigation started to the neglect or rejection of facts and inductions previously well established and the verification by abundant clinical observation. From this it will be seen special pathology is assigned for the junior, or better, the senior year, when the associated studies—medicine, autopsies and bacteriology—are well in hand and more profit

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gained by their co-relations, leaving clinical microscopy for the terminal part of the senior pathology, when students have access to the wards and can thus intelligently utilise the material and at the same time increase his laboratory knowledge. In closing, the question of examination of students is still much discussed, for the lecture is not sufficient and has many objections. The recitation method is only applicable to small classes, and for some students who are of a nervous temperament are not a fair test. The writing and correction method is excellent but not sufficient. Unless we can insure honest work, it is a severe tax on the teacher who corrects the papers and with slow writers does not give them a chance. It is a good plan to have a debate-day to teach the value and use of books and journals and so encourage a wider field of reading and consequent discussion, as well as to elicit many practical questions on not only the subject in hand, but its subdivisions and thus aid in showing a student the subject has a practical value and an intimate bearing with the sciences, most of which embrace the microscope. This, it seems to me, is the best method of teaching micro-science in medical schools.

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THE PRESIDENT'S ADDRESS.*

An Experimental Study of Aperture as a Factor in Microscopic Vision.

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It is a well known fact that the image of a point projected optically is not a point, but always a disc. The diameter of the disc varies directly with the uncorrected spherical and chromatic aberrations, and inversely with the aperture, of the projecting lens. If it were possible to fully correct spherical and chromatic aberrations, the image of a point would still be a disc varying in size inversely with aperture. Microscopists have long been familiar with the effect of spherical and chromatic aberrations in the microscope. But the effect of aperture as a factor in microscopic vision is not generally well understood and is worthy, I think, an hour's consideration by this society.

The practical importance of aperture in its relation to the resolving power of the microscope has been recognized longer than any explanation of that relation has been acceptable. Considered theoretically and independently as a factor in microscopic vision aperture has been almost ignored ; although as an associate factor, associated with diffraction by the finer details of microscopic objects, it has received no little attention. In fact, the final associated effects (in the projected image) of aperture and of diffraction of light in the plane of the object are the basis of the Abbe theory of microscopic vision. This theory and its easily repeated experimental study are fascinating and convincing. Since its announcement the Abbe theory has been for the most part accepted as rapidly as it has received serious attention. Nevertheless,

*Revised, extended and newly illustrated for the TRANSACTIONS of the Society.

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a few men* while appreciating the labors of Professor Abbe and recognizing the importance in some ways of his discovery have called attention to unsatisfactory points in the theory. The writer, if he may, will submit for consideration these and some other points and a theory of microscopic vision in harmony with an experimental study of aperture as a factor in microscopic vision.

The Abbe theory is said to be applicable only to microscopic vision, to the projection of an image by a microscope objective and not to image projection by a telescope objective or any other variety of projecting lenses. On the other hand, it is said that the theory applicable to the formation of an image by other projecting lenses is not sufficient to account for all the effects seen in an image projected by a microscope objective. Is not such exclusiveness, or limitation, unusual in the application of a theory to one particular function performed by essentially the same instrument (*i. e.* a projecting lens), although under somewhat different circumstances?

The writer believes that the theory of the effect of aperture as a factor in microscopic vision is applicable to all projecting lenses, explaining resolving power and its limitation; that the diffraction of light by an object is to be considered in the same category with other changes in direction in incident light produced by an object, as, for instances, those resulting from reflection and refraction; that diffracted and other rays leaving an object in changed directions, as well as rays directly transmitted, when traveling the same paths between an object and the objective are affected alike by aperture; and that the final effects in the image experimentally studied by Professor Abbe are the result of changes above the objective due to aperture, and not to changes below the objective resulting from diffraction by the finer details of an object.

A few years ago a portrait lens served the writer in showing the Abbe diffraction phenomena to a number of physicians met to listen to a paper on the theory of microscopic

*Particularly Mr. E. M. Nelson in *The Journal of the Quekett Microscopical Club*, July, 1890, and March, 1895; Mr. B. Tompson Lowne in the same *Journal*, April, 1889; and Mr. Lewis Wright in the *English Mechanic*, Vol. LX [1894].

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vision. The phenomena were the same as those with which most microscopists are familiar experimentally or by reading, and of which an authoritative account (*q. v.*) is given in the Dallinger edition of Carpenter's *Microscope and Its Revelations*. The portrait lens having a diameter of two inches and a focus of eight inches exhibited the phenomena on a large scale as well as a microscope objective exhibits the same phenomena on a smaller scale.

Early this month [August, 1896,] a parallel experiment was tried, using instead of the portrait lens a telescope objective having a diameter of two inches and a half and a focus of forty-three inches. An eye-piece was supported about sixty feet from the objective (*i. e.* the distance corresponding to tube length was about sixty feet, an actual tube of that length being unnecessary because the experiment was tried in dark rooms).. The source of light was an electric arc lamp about twenty-seven feet in front of the objective. The object was a series of vertical lines scratched with a fine needle point through the opaque film of an old dry plate negative supported nearly forty-six inches in front of the objective. After this gigantic microscope was focused so as to show the lines through the eye-piece, a plane was found not far from the eye end of the actual telescope tube in which was a central image of the electric arc with a series of diffraction images on each side. These images could be dealt with by means of diaphragms so as to vary the final image seen through the eye-piece as one deals with the corresponding images ["spectra"] at the back of the microscope objective to produce changes in the final image of the ordinary microscope.

The scratched lines in the latter experiment were about one-thousandth of an inch broad and from one-hundredth to one-sixtieth of an inch apart. The least of these measurements is more than a few multiples of any wave length of light. Since the publication of his original paper Professor Abbe has changed his views in respect to the size of objects to which the diffraction phenomena and the associated image

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changes are peculiar. He no longer holds that they are peculiar to objects measuring less than a few multiples of a wave length of light; for he has seen them produced by "gratings of not more than forty lines to the inch,"* seen through a lens of twelve inches focus.

If the Abbe diffraction phenomena and associated image changes can be experimentally demonstrated with a portrait lens or a telescope objective, is it not probable that they can be demonstrated with any projecting lens and, moreover, have any explanatory relation to the formation of the visual image in these cases that they have to the formation of the microscopic visual image? Again, if a portrait lens and a telescope objective behave in the same way as a microscope objective in experimenting with these diffraction phenomena and associated image changes, does a reason based upon such experiments exist for regarding microscopic vision as "*sui generis*"!†

There are three ways in which the projection of an image by a lens may be studied: One based upon the electromagnetic theory of stresses; a second, the physical, based upon the wave theory; and a third, the dioptric or geometrical, based upon the propagation of light in straight lines and the laws of reflection and refraction. The first has not been sufficiently studied to serve our purpose. The second, according to the Abbe theory, is the only one by which microscopic vision can be fully explained. The third which serves to explain the projection of images of macroscopic and larger microscopic objects is inadequate, according to the Abbe theory, to explain the projection of images of more minute microscopic objects.

A mixed method, largely the third and in part the second, is commonly used and is the "geometrical optics" of Lord Rayleigh and Professor Tait in the *Encyclopædia Britannica*. Under "geometrical optics" Lord Rayleigh not only includes "conceptions of the wave theory," but treats of "aperture"

* THE MICROSCOPE AND ITS REVELATIONS, Dallinger-Carpenter Edition, 1891, p. 64.
† *Ibid.*, p. 62.

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and "resolving power."* "Indeed," Lord Rayleigh says,† "it is not to be denied that the too rigid separation of optics into geometrical and physical has done a good deal of harm, much that is essential to a proper comprehension of the subject having fallen between the two stools." Under this interpretation of geometrical optics the writer believes that the projection of images of the more minute microscopic objects can be fully explained and, moreover, that such an explanation in some shape is a necessary part of a complete theory of the formation of images by every projecting lens.

For a time our discussion can be simplified by getting rid of the idea of the actual sizes of objects seen through the microscope or the telescope. When objects and distances between objects are measured not by linear units but by the angles they subtend at the center of the objective, "resolving power" in the microscope is seen to be the same as "separating power" in the telescope. Resolving power increases with "twice the sine of half the angle of aperture" of the microscope objective. Separating power increases with the diameter of the telescope objective. But twice the sine of half the angle of the aperture of a given lens is its effective diameter. The same power, then, is required to show as separate pictures the images of two points of detail subtending an arc of one minute whether the actual distance between the points be very little, measured on the stage of the microscope, or very great measured among the stars. Resolving power may be regarded as separating power brought to bear upon an object near a microscope objective and separating power as resolving power brought to bear on an object a great distance from a telescope objective.

In Fig. 1, let A and B be isolated centers of motion of equal intensity radiating yellow light. Let SN be a section of a screen interrupting light from the points A and B. Let the rays of light Ac and Bc be equal in length. Let the ray Ad be shorter than the ray Bd by half a wave length of yellow

* Encyclopædia Britannica, Ninth Edition, Vol. XVII., p. 806.
† *Ibid.*, p. 798.

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light, Ae a whole wave length shorter than Be, Af a wave length and a half shorter than Bf, and Ag two wave lengths shorter than Bg. At c, e and g where whole waves of the incident rays coincide crest with crest and trough with trough the intensity of one ray is added to the other. At the intermediate points d and f where crests oppose troughs and troughs oppose crests motion becomes null and darkness results. The increased intensity at c, e and g is not limited strictly to these points in the line SN but is spread out upward and downward to a certain extent and then fades into the intervals of darkness. This is because crests nearly coincide with crests at first, when the difference in the lengths of the uniting rays is still nearly a wave length or an integral multiple of a wave length. But as waves fail more and more to coincide crest with crest and trough with trough and tend more and more to oppose troughs to crests and crests to troughs, interference results in less and less motion and consequently less and less light.

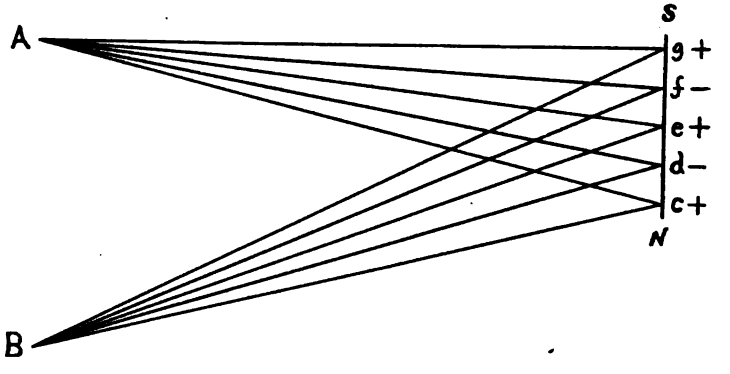

FIG. 1.

The distances between the points of greatest intensity c, e and g are seen to depend upon the wave length of yellow light. If the centers A and B radiated red light, with a longer wave length, the distances between the points of greatest intensity on the screen would be greater and the upward and downward spreading of light would be more

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correspondingly. On the other hand, if the centers *A* and *B* radiated blue or violet light, with a shorter wave length, the distances between the points of greatest intensity would be less and the upward and downward spreading of light reduced correspondingly.

If the screen were nearer or farther from *A* and *B*, it would present the same appearance with this exception; that the distances between the points *c*, *d*, *e*, *f* and *g* would vary with the distance between the sources of light and the screen. If the line *SN* of the screen were longer, additional points of darkness and light would appear above *g* and below *c*. [In this and the diagrams about to be used the wave length is thousands of times longer than an actual wave length of light; so the distances between such points as *c* and *d*, or *c* and *e*, are thousands of times too great, made so for purposes of demonstration.]

In Fig. 2, let *ABCDE* be a section of the emitting surface of a projecting lens. Let *O* be a self luminous object (or one illuminated with a full cone of sub-stage light) subtending a small angle, *bCc*, at the center of the lens. Let *OA*, *OB*, *OC*, *OD* and *OE* be rays which radiate from a central isolated point in the object *O*, pass through a first lens surface not shown and reach the emitting lens surface *AE*. Let *SN* be a section of a screen receiving the projected image of a central isolated point in the object *O*. In addition to the refracted primary rays *Ag*, *Bg*, *Dg*, *Eg* and the direct primary ray *Cg*, other less intense secondary rays*—such as those shown by broken lines—are also propagated from the lens to the screen, the secondary rays having less intensity with their obliquity to the primary ray from which they radiate. Such radiating secondary rays originate at every emitting point of the lens surface *AE*. Secondary rays from two isolated points, as *B* and *C*, when interrupted by the screen *SN*, would behave as behaved the radiating rays in Fig. 1.

The full aperture of the lens is *AE*. Suppose this aper-

*For an account of the origin and behavior of secondary waves, or rays, the reader is referred to Preston's THEORY OF LIGHT, Chapters III, VII, VIII and IX.

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ture to be reduced one-half, the emitting surface AE reduced to BD. Thus the points of emission A and E are excluded by this first supposition. Let B, C and D be isolated points of emission, B and D being at opposite edges of the aperture.

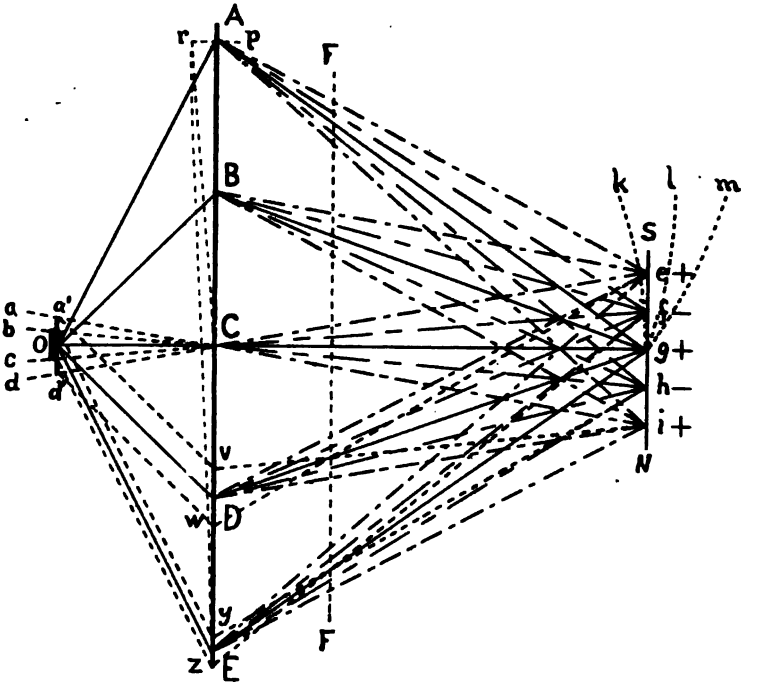

FIG. 2.

Let the rays Bf and Dh be longer than the rays Cf and Ch by half a given wave length. Let the rays Be and Di have the same length as the rays Ce and Ci. Let the ray Bi be longer than the rays Ci and Di by two wave lengths and the ray De be longer than the rays Ce and Be by two wave lengths. As a result, the points e, g and i in the line SN are illuminated and the points f and h are dark. Spreading of light occurs at e, g and i. If the screen were more extended, additional points of light would be seen above e, and below i. What occurs in the line SN, one diameter of the screen, occurs in all diameters. Consequently g becomes

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a disc of light with a fading peripheral zone while *f* and *h* become a ring of darkness and *e* and *i* a ring of light. If the screen were more extended, larger and concentric rings of darkness and light would be added to the pattern. The intensity of the smallest ring of light would be much less than that of the center of the disc, and with increasing diameter the intensity of each ring would rapidly diminish to invisibility.

If, now, the object *O* be an unbroken surface and self luminous (or illuminated with a full cone of sub-stage light), lying between the dotted lines *bC* and *cC*, then its diameter would be made up of a line of luminous points each of which would radiate light to the projecting lens as did the central point we have considered. One ray from each point would pass directly through the center of the lens and fall upon the screen *SN* somewhere between the points *f* and *h*. Each of these direct primary rays would at its point of emission *C* in the lens surface *BCD* give origin to radiating secondary rays. One ray also from each point would after refraction by the lens leave the emitting surface at *B* and fall upon the screen somewhere between the points *f* and *h*. Each of these refracted primary rays would at its point of emission *B* give origin to radiating secondary rays. So, also, corresponding primary refracted rays would be emitted from *D* and fall upon the screen between *f* and *h*, each giving origin on emission to similar radiating secondary rays. Then might *Ce* be regarded as a secondary ray originated at *C* from a primary ray in the path *cCf*, and *Ci* a secondary ray from a primary ray in the path *bCh;* and *e* would now have the same relation to *f*, and *i* to *h*, as we saw a moment ago *f*, or *h*, had to *g*. *f* and *h* would now be bright ends of the image *fh* of the diameter of the object *O;* and the points *e* and *i* would be dark by interference, while spreading of light from *f* toward *e*, and from *h* toward *i*, would occur. This spreading of light from *f* upward and from *h* downward results in elongating the image of the diameter of the object. [A perfect dioptric image would be sharply brought to an end at *f* above

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and *h* below.] Were the screen *SN* a little more extended downward, a bright point would appear below *i* and would have the same relation to *h* that we saw a moment ago *i*, or *e*, had to *g*. A corresponding point would appear above *e*, if the screen were extended a little upward. What would be true in the projection of an image of one diameter of an object would be true in the projection of an image of every other diameter. Consequently, the surface of the object *O*, supposed to be round, would be projected on the screen *SN* as a disc having a diameter a little greater than *fh* and not a sharp but a fading, or fluffy, boundary. Were the object a line and *O* between *bC* and *cC* its section, then the image would be a band of which the diameter image already described would be a section. Were the screen more extended, the disc would have concentric rings about it, or the band would be flanked above and below by parallel lines. (See first the dots and lines *a*, *i* and *g* in Photo. 1, and then the same dots and lines in Photo. 6.)

It will be observed that in the first instance (the projection of an image of a central isolated point) *e* and *i* were bright and in the second (the projection of an unbroken surface) dark. In either case the illumination or darkness was the effect of the disturbance at *e* or *i* resulting from the union of the wave motions of all the rays there incident. If in the first instance all the points in the diameter *BCD* were simultaneously effective, the illumination at *e* would be the result not only of the union of coincident crests and troughs of the secondary rays *Be*, *Ce* and *De*, but also of a like union, pair by pair, of one secondary ray from each succeeding point of emission in the lens surface downward from *B* to *C* with one secondary ray from each corresponding succeeding point of emission downward from *C* to *D*. The illumination at *i* would be the result of a similar union of the secondary rays *Bi*, *Ci* and *Di* and also the union, pair by pair, of secondary rays from the points between *B* and *C* with secondary rays from points between *C* and *D*. (A primary ray is indicated by an unbroken line, as *Cg* or *Bg*; a secondary ray of first intensity,

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by a broken line of long portions, as *Bf* or *Bh*; and a secondary ray of second intensity, by a broken line of shorter portions, as *Be* or *Bi*.

If in the second instance all the points in the diameter *BCD* were simultaneously effective, the darkness at *e* would be the result not only of the union of *Be*, *Ce* and *De*, opposed in phase, but also a like union, pair by pair, of one secondary ray from each succeeding point of emission in the lens surface downward from *B* to *C* with one secondary ray from each corresponding succeeding point of emission downward from *C* to *D*. Darkness at *i* would be accounted for in a similar way. What would be true in this and the former instance in one diameter would be true in all diameters of the same surface.

Each lens surface in an objective gives rise to secondary rays and is also a reflecting as well as a refracting surface. So the primary rays incident at the last surface of an objective are ray by ray the compounded resultants of the union of direct, reflected and secondary rays (less energy by giving rise to secondary rays and by reflection) at all previous transmitting surfaces. The total result in a path leaving the last surface is compounded wave motion which on being interrupted by a screen unites with other resultant compounded waves arriving by different paths at the same point in the screen. But the primary rays in their paths through the objective are affected essentially alike; so that those which leave any one point in an objective arrive at the image of that point in the same phase. A primary ray determines the phase of the secondary rays to which it gives origin at the last surface of the objective. Thus the phase of the compounded wave motion of the ray *Be* is determined in the second instance chiefly by the phase the primary ray *Bf* at the emitting point *B;* the phase of *Ce*, chiefly by that of *Cf* at *C;* and the phase of *De*, chiefly by that of *Df* at *D*.

Now let the full aperture *AE* in Fig. 2 be effective. Let an image of a central isolated point of the object *O* be projected by the lens upon the screen *SN*. By the doubling of aperture, the lens not only receives additional and more

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oblique rays from the object, but has additional points of emission. *OA* is one of the additional and more oblique rays from the object. *A* and *E*, at opposite edges of the double aperture, are additional points of emission. After refraction the ray *OA* takes the path *Ag*, which is evidently longer than the path *Bg* taken by the ray *OB* after refraction. Both these paths are longer than the path *Cg* taken by the ray *OC* after transmission.

Let the arc *gm* be drawn with *Ag* as a radius; the arc *gl*, with *Bg* as a radius; and the arc *gk*, with *Cg* as a radius. If the *g* ends of the first two radii were swung upward in the arcs *gm* and *gl*, the interrupting screen *SN* would shorten the radius *Ag* more rapidly than it would the radius *Bg* (relatively as are the distances of the arcs *gm* and *gl* from the screen *SN*). If the *g* end of the radius *Cg* were swung upward in the arc *gk*, it would have to be longer to reach the screen. This addition in length to enable it to reach a given point in the screen would be less than the shortening the radius *Bg* or *Ag* would suffer if interrupted at the same point. It becomes evident diagrammatically that were the *g* ends of these radii swung upward in the described arcs, the difference in length between the radii *Ag* and *Cg* would amount to half a wave length in a swing about half as great as the swing it would be necessary to give the *g* ends of *Bg* and *Cg* to make the latter differ in length by half a wave length. As a result, such darkness as the aperture *BD* placed at *f* would, by the double aperture *AE*, be placed half way between *g* and *f*. In a similar way, such illumination as the smaller aperture placed at *e* would, by the larger aperture, be placed at *f*. In a corresponding way, the spreading of light upward from *g* would be reduced to half what it was with the half aperture. Symmetrical phenomena would occur below the axis of the lens. What is true of one diameter of the screen is true of all its diameters. Therefore, the image of a central isolated point in an object is projected by a lens of given aperture as a disc and concentric rings having diameters equal to half those of the disc and rings in the image projected by a lens of half

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the given aperture. A similar study of other apertures would but add to this evidence showing that the interference or diffraction pattern, disc and rings, is proportionally contracted with every increase of aperture.

Not only have microscopists noticed in practice the direct relation of aperture to resolution, but also the fact that isolated lines or particles in an object appear broader through an objective of small aperture and narrower through an objective of large aperture. This narrowing effect of increasing aperture is due to the contraction of the diffraction pattern. *It is easily understood that the projected image discs of a series of close points in an object (or the projected image bands of a series of close lines) might touch or overlap when projected by a lens of small aperture, and, on the other hand, might be separated or resolved when projected by a lens of sufficiently large aperture.* The separating or resolving power of the telescope is thus explained. The same explanation has been applied, by inference at least, to projecting lenses generally, not excluding the microscope. Only Professor Abbe and his followers exclude the microscope and claim that microscopic vision is "*sui generis.*"

Simple parallel experiments with the telescope and microscope show that the actual effects of aperture in both instruments are in harmony with the above explanation:

Experiment 1: The instrument used was a telescope having an aperture of two inches and a half and a focus of forty-three inches, standing twenty-seven feet from a window in a darkened room. Outside the window was a mirror reflecting light from a bright sky into the room. Of all the light reflected from the mirror that only reached the telescope which passed through two pinholes in a piece of black paper supported in front of the mirror. The diameters of the pinholes were one-thirtieth and one-twentieth of an inch respectively; and the distance between them, one-tenth of an inch. The iris diaphragm of an Abbe sub-stage condenser was supported centrally in a temporary mounting of wood fitting into the hood of the objective.

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When the diameter of the iris opening was one-sixteenth of an inch, the two pinholes appeared, when seen through the telescope, as one dim hazy disc. When the diameter was one-eighth of an inch, a smaller and more distinct disc was seen. When the diameter was three-sixteenths of an inch, the disc was still smaller, brighter and better defined—with a dim, hazy overlapping disc becoming evident. When the diameter was one-fourth of an inch, the discs (now distinctly two) were smaller, brighter and just separated. When the diameter was three-eighths of an inch, both discs were brilliant and well separated, their relative sizes and distance apart approaching truth. When the diameter was one-half an inch, the picture was more brilliant—the larger disc tending to appear star-like with irradiation. With the full aperture of two inches and a half irradiation was marked in both. During these observations thin concentric circles of light were glimpsed.

Experiment 2: The instrument and all the conditions excepting one remained the same. The exception was this: in the hood was fitted a piece of stiff black paper instead of the iris diaphragm, the circular piece of paper allowing no light to enter the objective except that which passed through a slot corresponding with one of its diameters. Thus the objective was made rectangular in shape with a narrow aperture in one direction and a long, or wide, aperture in the other.

The piece of paper with its slot was turned about so that the slot corresponded with various diameters of the hood. The discs seen through the telescope appeared stretched out, as it were, into lines always crossing at an angle of 90° the diameter of the instrument corresponding with the slot. The width of each line was determined by the long aperture; the length, by the narrow aperture. A comparison of the width of each line with its length showed the comparative effect of the two apertures in contracting the diffraction pattern.

Experiment 3: The instrument used was a Powell and Lealand microscope with a centering and focusing sub-stage. The objective was a Zeiss "aa", having a focus of about 1-inch, and N. A. of .17. The eye-piece was usually a Powell

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and Lealand "compensating 20." The sub-stage carried a Powell and Lealand apochromatic condenser of $\frac{1}{4}$-inch focus, and N. A. of about .9. At a distance of three feet in front of the microscope the same pinholes used in the first two experiments were arranged so as to allow only such light from a lamp flame as passed through them to reach the mirror of the microscope. The light reaching the mirror was reflected through the sub-stage condenser to an aërial image of the pinholes projected by the condenser in the plane of the microscope stage.

The aërial image of the pinholes was the object observed through the microscope. Seen with a small diaphragm opening back of the objective the pinholes appeared as two discs just touching one another. With larger openings the discs became smaller, more brilliant and separated. The effects of varying aperture (varied by means of diaphragm openings back of the objective) in this experiment with the microscope were the same as those seen in the first experiment when aperture was varied by means of diaphragm openings in front of the telescoe objective.

Experiment 4: The instrument and all the conditions excepting one remained the same as in the third experiment. Instead of the central opening in a diaphragm back of the objective, a slot corresponding to one diameter was used. The aperture of the objective thus became rectangular in shape.

The image of the pinholes was observed while the slot was turned so as to lie successively in all diameters of the instrument. The effects were the same as those seen when the corresponding Experiment 2 was made with a telescope. Before the diaphragm was placed back of the objective the discs appeared as shown diagrammatically in Fig. 3 at a and b. Later, with the slot directed as shown at A, the image became a line for each disc (a' and b') with diffraction points at each end, the latter not shown in the figure. With the slot directed as shown at B the diffraction patterns overlapped (a'' and b''). a' and b' show resolution by wide aperture (the

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length of the rectangular aperture); and a'' and b'', failure in resolution by narrow aperture. With a slot three times as wide the lines a' and b' become one-third as long; and a'' and b'' did not overlap, but appeared as shown at a''' and b'''. Resolution in the last instance was affected by both apertures, but to a greater degree by the longer.

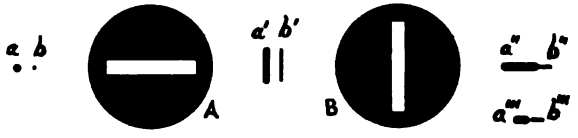

FIG. 3.

Resolution and failure in resolution are also shown at H and D in Photo. 25, taken with a horizontal slot, and at H and D in Photo. 26 taken with the same slot placed vertically, the object and other conditions in each case being the same. In these two Photo's are seen faint diffraction points at the ends of some lines [well shown in the photomicrographs, but not in the half-tone reproductions] and faint diffraction lines flanking others, depending upon the direction of the slot.

Our geometrical and experimental study thus far suggests the following:

A perfectly projected dioptric image [one free from defects due to spherical and chromatic aberrations and diffraction] would be point by point a correct picture of an object. But as the images of points are not projected as points, but as discs, a perfectly projected dioptric image is an impossibility. Excluding spherical and chromatic aberrations, these discs (in so much that they are larger than points) are due to aperture diffraction effects. Aperture diffraction effects are contracted with every increase of aperture. *The essential object of increasing aperture is to contract diffraction patterns, and thus cause the projected image to approach as a picture as nearly as possible that which we can only imagine, never realize, a perfect dioptric image.* The more nearly the picture approaches in character a dioptric image, the less will be the overlapping of the projected images of details, the greater will be the

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separating or resolving power of the projecting lens, and the better will be our idea of the object under observation.

This is illustrated by Photo's 9 and 10. Photo. 9 was taken with an aperture barely wide enough to resolve the closer of a double series of lines cut through an opaque film of silver. The bare resolution of the closer lines gives us a poor idea of fine cuts in an opaque film. We need to see the details of the edges of the lines and of appearances between the lines (as in Photo. 10), requiring wider aperture in various diameters, to get a better idea of cuts and imperfections in the film. *We need then wider and wider aperture to resolve details of details, and wider and wider aperture still in every diameter to resolve successively finer and finer details of details (as long as they are present, or a practical limit to increase of aperture is not reached). This is accomplished by contracting more and more the diffraction disc image of each of the individual points of which every object may be considered to be an aggregation, be its form what it may.* Not only does our study thus far suggest the foregoing theory of microscopic vision, one applicable to all projecting lenses, but calls attention to the fact that one of the essential factors of the Abbe theory is not always present; for *the details of our self-luminous aërial microscopic object originated no diffracted rays*, although the microscope objective projected and resolved in usual fashion.

Suppose, for a moment, our aërial object originated diffracted rays. As every path between the object and the objective was occupied by a primary ray, diffracted rays could have taken only the same paths. Starting from the same points in the object they could have had no individuality; for each, diffracted from one primary ray, would have become, at its very origin, a part of another primary ray leaving the same point—just making up for the loss by the latter of a corresponding diffracted ray given to the former. A similar exchange of diffracted rays everywhere* in the cone would

*"Everywhere" is not strictly true when a cone just fills an objective. Primary rays toward and at the periphery would then lose rays diffracted outward beyond the limit of the cone and would receive no diffracted rays in return, as there would be no primary rays outside the cone from which diffracted rays could originate.

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have resulted in primary rays not differing appreciably from those which occupied the same paths between the object and objective, diffraction not occurring. Such were the conditions under which Photo's 9 and 10 were taken. The irregular rectangular figure [the same as *B* in Photo. 1] is the area of the source of light projected in the plane of the film of silver, projected by a sub-stage condenser cone of light of more than sufficient angle to send primary rays along every path between the object and the objective. Diffraction by the cuts and imperfections in the film resulted in an interchange of diffracted rays, but no appreciable alteration in the character of the primary rays, *i. e. diffracted rays from an object lose individuality at their very origin when an objective is filled by a full cone of light from a sub-stage condenser.* Under second conditions then we find a factor essential to the Abbe theory of microscopic vision is not necessary ; *for Photo's 9 and 10 show the effects of different aperture notwithstanding diffraction by the details of the object was practically nullified by full cone illumination.*

That rays diffracted by an object are practically nullified by full cone illumination can be shown experimentally. While the apparatus was arranged for taking Photo. 9, the full cone of light from the sub-stage condenser was reduced to a small axial pencil. A bit of white card was held against the front of the objective. Each cut in the film diffracted light, spreading obliquely away from the axial pencil. The diffracted rays leaving different cuts and becoming incident at any one particular point on the card traveled paths of different lengths. Interference phenomena occurred. Any pair of adjacent cuts had essentially the same relation to the card that the two sources of light *A* and *B* in Fig. 1 had to the screen *SN*. The result on the card was a bright round spot of light (illuminated by the axial pencil) flanked on either side by fainter repetitions of the round spot corresponding in their manner of production with *e* and *g* in Fig. 1. The fainter spots were formed wholly by diffracted rays from the cuts. A full cone from

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the sub-stage condenser was then used again. Primary rays once more traveled every path between the cuts and the card, and engulfed, as it were, the diffracted rays. The card was illuminated by one large circular spot, including and blotting out all the previous picture.

Lord Rayleigh, writing of resolving power of optical instruments in general, and of the telescope in particular, says: "The contraction of the diffraction pattern with increase of aperture is of fundamental importance with reference to the resolving power of optical instruments"*; resolving power is "proportional to the aperture, and independent of focal length"†; and "The theory of resolving power is rather simpler when the aperture is rectangular instead of circular, and when the subject of examination consists of two or more light or dark lines parallel to one of the sides of the aperture. Supposing this side to be vertical, we may say that resolving power is *independent of the vertical aperture*, and that a double line will be about on the point of resolution when its components subtend an angle equal to that subtended by the wave length of light at a distance equal to the *horizontal aperture*."‡ Lord Rayleigh§ is also authority for the experimental result that, if for a rectangular aperture under the conditions just quoted a round aperture be substituted, the latter to have the same resolving power must have a width or diameter about ten per cent greater. Sir George Airy¶ has found by calculation that under the same conditions the width or diameter of a circular aperture must be about twenty per cent greater. Here is disagreement as to the exact difference, but not as to the fact that of two apertures of the same width or diameter, one rectangular and the other round, the resolving power of the rectangular aperture is greater.

We have dwelt upon the "fundamental importance" of the contraction of the diffraction pattern with increase of aper-

* ENCYCLOPÆDIA BRITANNICA, ninth edition, Vol. XXIV, p. 430.
† *Ibid.*, Vol. XVII, p. 807. ‡ *Ibid.*
§ *The Journal of the Quekett Microscopical Club*, March, 1895, p. 28 (Nelson).
¶ *Ibid.*, p. 27 (Nelson).

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ture with reference to the resolving power of the microscope, and the fact that resolving power in this instrument is proportional to its aperture. Your attention is now invited to the other optical laws quoted, though not in their order of quotation. "A double line will be about on the point of resolution when its components subtend an angle equal to that subtended by the wave length of light at a distance equal to the horizontal aperture," when aperture is rectangular and the double line is parallel with the vertical sides. Now, suppose *BD* in Fig. 2 to be the width of a rectangular aperture. By comparing the lengths of lines we have already considered in this diagram we find the arc *rp* is equal to two wave lengths of light. *AC* is equal to *BD*. Therefore, the angle *ACr* is the angle a wave length (half of the arc *rp*) subtends at a distance equal to the aperture *BD*. This angle on comparison is found to be half as great as the angle *bCc* and *fCh*. Accordingly, lines separated by an angular distance equal to half that between the lines *bC* and *cC* would be about on the point of resolution by an aperture equal in width to *BD*.

The aperture *BD** in Fig. 2 projects a central point in an object as a disc having a diameter of which the radii extend upward from *g* half way toward *f* and downward from *g* half way toward *h*. The diameter of this disc therefore subtends at the center of the objective *C*† half the angle *fCh*, or half the angle *bCc*, an angle equal to the angle *ACr*, or to the angle subtended by a wave length at a distance equal to the aperture. If a point in the object *O* at the crossing of the line *bC* be projected by the same aperture *BD*, its image will be a disc with *h* as its center, and an upper radius extending half way toward *g* where it would about meet the lower end of the diameter of the first disc. The two discs would about touch. If a point in the object *O* at the crossing of the line *cC* be projected by the same aperture, its image will be a disc with *f* as its center, and a lower radius extending half way toward *g*, where it would about meet the upper end of the

* Supposing the resolving power in each diameter of the aperture be the same as that of a rectangular aperture of equal width [also true, for simplicity, in previous use of Fig. 2]

† *C*, in Fig. 2, is both the center of the emitting surface and the center of the objective.

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diameter of the first disc. These three discs on the screen about touching one another would be a picture of three points in the object about to be resolved. The centers remaining the same, lessening the diameter of the discs would result in a picture made up of three discs not touching one another; and, consequently, the corresponding three points of detail in the object would be resolved.

Now, let AE be the width of a rectangular aperature. At a distance equal to this double aperture a wave length, half of rp, would subtend the angle AEr. The angle AEr is evidently equal to half the angle ACr. Accordingly the resolving power of the double aperture should be twice as great as that of the aperture BD (equal to AC). We have already found [p. 332] this to be true: that the image discs of points have half the diameter when projected by this double aperture (as compared with image discs projected by half that aperture), and that, therefore, the resolving power is twice as great. We thus find our diagram in harmony with the law last quoted, supposing the aperture to be rectangular.

As to the difference in resolving power of a rectangular and round aperture: Lord Rayleigh, experimenting with the telescope, found a round aperture should have a diameter a little less than ten per cent greater than the width of a rectangular aperture to give the same resolution; while Sir George Airy, by calculation, found a round aperture should have a diameter a little more than twenty per cent greater than the width of a rectangular aperture to give the same resolution. To determine which result is nearer right practically in reference to resolving power in the microscope, I ruled the lines shown [not accurately reproduced] in Fig. 4; and photographed aërial images of them by means of square and round apertures. The lines were ruled on a Geneva Society dividing engine [in the physical laboratory of a kind friend, Professor E. Haanel, of Syracuse University], ruled in or cut through the opaque film of an Eastman gelatino-bromide lantern plate, exposed [to a gas jet for a few seconds], developed,

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fixed, washed and dried in the usual way. The numbers indicate the distances between the lines in millimetres.

An aërial image of the lines was projected with a sub-stage condenser of $\frac{1}{4}$-inch focus. This image in the plane of the microscope stage was photographed with a Wales $1\frac{1}{2}$-inch objective, and a Powell and Lealand "compensating 20" eye-piece. Photo. 11 was taken with a round aperture of $5\frac{1}{2}$ mm.; Photo. 12, with a round aperture of 6 mm.; and Photo. 13, with a square aperture of 5 mm. (the sides being parallel with the lines). An aperture of $5\frac{1}{2}$ mm. is ten per cent greater, and an aperture of 6 mm. twenty per cent greater, than an aperture of 5 mm. Then, according to Lord Rayleigh, resolution should be nearly the same in Photo's 11 and 13—a trifle better in Photo. 11. According to Sir George Airy, it should be nearly the same in Photo's 12 and 13—a trifle better in Photo. 13. [In Photo's 11 and 13 the group of lines marked .*46* should be shown barely resolved, resolution being slightly more evident in Photo. 11. Lines marked .*42* in Photo's 11 and 13 should show no resolution, while the same lines in Photo. 12 should be shown resolved slightly better than are the lines .*46* in Photo. 13—or resolved as well as the lines .*46* in Photo. 11. These differences shown in the photomicrographs are hardly perceptible in the half-tone reproductions.] If the widths of the alternating white and gray bands [the latter of which should be black] under *1*, in Photo. 11, be critically compared with the corresponding bands in Photo's 12 and 13, it will be seen that the gray bands are broadest in Photo. 12, and of nearly the same breadths in Photo's 11 and 13. Corresponding results were got in other photomicrographic experiments.

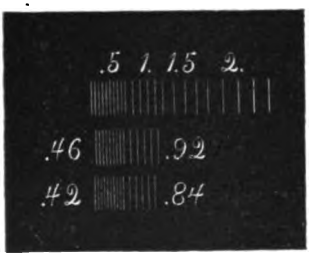

Fig. 4.

It will be noticed that the closeness of the .*46* mm. group of lines is about ten per cent greater, and that of the .*50*

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mm. group about twenty per cent greater, than that of the .42 mm. group. The closeness of the .92 mm. group of lines is about ten per cent greater, and that of the 1. mm. group about twenty per cent greater, than that of the .84 mm. group. Such gradation in closeness made comparison of the results of photomicrographic experiments an easy matter. The results were found to agree with Lord Rayleigh's experiments and not with Sir George Airy's calculation.

That resolving power is "independent of focal length" when distances between details of objects are measured by the angle they subtend at the center of the projecting lens becomes evident on again studying Fig. 2. Suppose the focal length of the lens, a little less than *CO* in the diagram, to be only half as great, the screen *SN* remaining fixed. Then the object to be projected on the screen would be necessarily half as far from *C* as *O* is from *C*. The round surface *O* would require only half as great a diameter in the new position to be projected to the same pattern on the screen, by the same aperture. The lines of the diagram would have to be changed only by shifting the *O* ends of *OA*, *OB*, *OC*, *OD* and *OE* to a point half way between *O* and *C* to enable us to use Fig. 2 for demonstrating the projection of a round object having half the diameter of *O* by the lens having half the focal length. The distance between the points in the lines *bC* and *cC* touching the ends of the vertical diameter of *O* in its new position would be half that between the points in the same lines which touch the ends of the diameter in the present position of *O*. The angle subtended at *C* by the diameter of *O* in its present position is *bCc*. This is the same angle that *O* with half as great a diameter in the new position would subtend at *C*. Thus Fig. 2 would show resolving power to be "independent of focal length" when measured by the angle the distance between details subtends at the center of the projecting lens. That this optical law applies to both the telescope and the microscope [or to any other projecting lens], is what one would expect, bearing in mind the fact that a

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telescope becomes a microscope by shortening sufficiently the longer of its conjugate foci, and a microscope a telescope by lengthening sufficiently the shorter of its conjugate foci.

While resolving power is independent of focal length when measured by angles subtended at the center of the projecting lens, it is not independent of focal length when measured by the actual distances between details subject to resolution. This truth, set aside early in our study to simplify discussion, may here be considered. We have just seen that *O* in Fig. 2 would require only half as great a diameter to be projected to the same pattern on the screen *SN*, if the lens had half as great a focal length. Therefore, what was said in a preceding paragraph [page 340] regarding the projection of three points of detail in the object *O* would be true of three points of detail half as far apart in the smaller object, half way between *O* and *C*, projected on the screen *SN* by a lens of half as great a focal length. Then, in turn, neglecting the angles subtended at the center of the projecting lens by distances between the details of an object, we find that resolving power relating to actual distances in an object projected by the same aperture varies inversely with focal length. With the same aperture, a $\frac{1}{2}$-inch objective would resolve a series of lines half as far apart as would a 1-inch objective.

Photo. 15, of an aërial image of the ruling shown in Fig. 4, was taken with a $\frac{1}{2}$-inch objective having an aperture of 2 mm.; and Photo. 16 of the same aërial image, with a 1-inch objective having an aperture of 2 mm. Different eye-pieces were used so as to get nearly the same amplification. The relative closeness of the lines is shown in Fig. 4. Compare the lines *1* unit apart in Photo. 15 with those *2* units apart in Photo. 16. Compare these groups with the others in the two Photo's as to the comparative width of lines and the intervening spaces. Of all the lines in Photo. 16 those *2* units apart are, lines and interspaces, most like those *1* unit apart in Photo. 15 (the interspaces between the lines *1.5* and *2* units

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apart in Photo. 15 being comparatively too wide). There is additional evidence in the photomicrographs [not seen in the half-tone reproductions], showing that with the same aperture resolving power varies inversely with focal length when distances between the details are subject to linear instead of angular measurement.

The two expressions regarding the relations of resolving power and focal length are, however, quite in harmony. While resolving power measured by angles, at the center of the projecting lens, subtended by details in an object is independent of focal length, a shorter focal length (for instance) necessitates a shorter distance between the object and the objective. This results, on the one hand, in the same details subtending at the center of the projecting lens a proportionately greater angle; or, on the other hand, proportionately less separated details subtending the same angle. In so far as the details subtending the same angle are less separated as a result of shorter focal length causing the object to approach the objective, resolving power relating to actual distances in the object is proportionately increased.

We have seen that the resolving power relating to actual distances between the details of an object [and this is the common meaning of resolving power in the microscope] is, first, inversely proportional to the angle (at the center of the projecting lens) a wave length of light subtends at a distance equal to the aperture. Secondly, it is inversely proportional to the focal length of the projecting lens. This means (referring again to Fig. 2) that by decreasing the angle ACr, or by causing the object O to approach the lens, resolving power is increased. Practically, there are limits to these two operations, and hence a limit to resolving power.

Let us see how these limits apply. It is practicable to cause an object to so far approach a lens that the object all but touches the glass, while the objective takes in from the object a cone of light having an angle of very nearly 180°. In such an instance a limit to resolving power is practically reached. The object could not be nearer the objective;

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and greater aperture could not be utilized, because the cone of light from the object reaches approximately the highest possible angular limit. But such a limit reached in the case of a dry objective used with white light can be passed, and is passed, in the case of an immersion objective, or a dry objective used with light of shorter wave length. Consider for a moment the use of monochromatic blue light of shorter wave length: As with such light the arc *rp* in Fig. 2 would be shorter, and the angle *ACr* proportionately less, resolving power—varying inversely with this angle—would be greater. Increase of resolving power with the use of blue light is recognized in practice, particularly in photomicrography. With the use of light of shorter wave length the angle *ACr* is lessened by shortening the subtending arc *rA*. With the use of immersion objectives this angle is lessened by increasing *AC*, or the diameter of the emitting surface *BD*.

In Fig. 5, let *O* be a point in an object and *L* the front lens of a dry objective. Let *aOb* be a cone of light leaving *O* and entering the lens *L*. Suppose the ray *Oa* to be refracted by the surface *vw* so as to reach the surface *xz* at *c* and the ray *Ob* so as to reach the surface *xz* at *d*. The diameter of the emitting surface is then *cd*. Now, suppose *O* and the cone *aOb* to be in a fluid having the same index of refraction as the glass of the lens *L*. Then the rays *Oa* and *Ob* would suffer no change in direction on entering the lens, *Oa* reaching the surface *xz* at *m* and *Ob* the same surface at *n*. The diameter of the emitting surface would now be increased by the difference between *cd* and *mn*. The greater the index of refraction of the lens *L* the greater would be the increase of aperture by homogeneous immersion. Suppose the lens *L* to have a higher index of refraction. Then a cone of light in air of greater angle, *vOw*, might after refraction by the surface *vw* be emitted by the surface *d*. Now, suppose *O* and the cone of light *vOw* be in a fluid

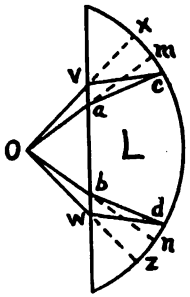

Fig. 5.

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having the same index of refraction as the glass of the lens *L*, with its higher refracting power. The rays *Ov* and *Ow* would suffer no change in direction on entering the lens, *Ov* reaching the surface *xz* at *x* and *Ow* the same surface at *z*. The diameter of the emitting surface [or effective aperture] would now be increased by the difference between *cd* and *xz*.

A few lenses have been made of glass of exceptionally high refractive index, requiring a special homogeneous immersion sub-stage condenser, stage slips and covers made of special glass, as well as an immersion fluid of correspondingly high refractive index. Such lenses and necessary accessories made by Zeiss, of Jena, are very costly and very limited in usefulness, while the gain in aperture is only about 60 per cent (over dry apertures) as compared with the gain of about 50 per cent in ordinary homogeneous immersion objectives.

The estimating of resolving power as to actual distances in a microscopic object is a complicated matter ; for, in addition to aperture as a factor, we have found a consideration of the effects of focus and immersion fluid must be included. The meeting of this difficulty by Professor Abbe conferred a boon upon microscopists. By means of Professor Abbe's "numerical aperture"* [expressed by N. A.] it becomes a comparatively easy matter to ascertain the resolving power of microscope objectives. Numerical aperture tables, exhibiting the theoretical limit of resolving power under different conditions of wave length, immersion fluid and aperture, are now widely accessible, for instance in the Dallinger-Carpenter edition of *The Microscope and Its Revelations*, p. 84. These tables, however, are based upon the Abbe theory and, therefore, exhibit too high a limit—that is under conditions suitable for best microscopic vision [a matter to be considered later].

A study of the effects of projection by zonular apertures is not only interesting but of practical importance ; because

* For an explanation of "numerical aperture" the reader is referred to Chapter II in the Dallinger-Carpenter edition of THE MICROSCOPE AND ITS REVELATIONS. In this paper aperture means opening, or extent of opening measured by its diameter or breadth.

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Fig. 6.

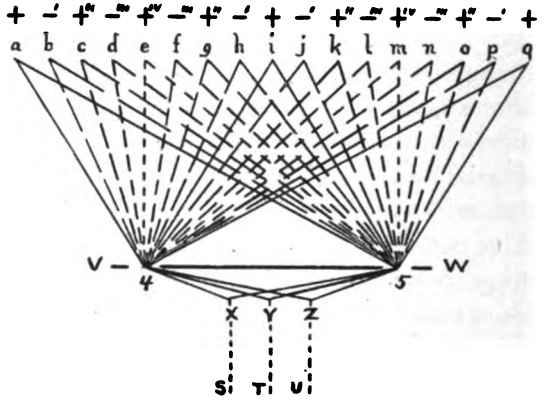


Fig. 7.

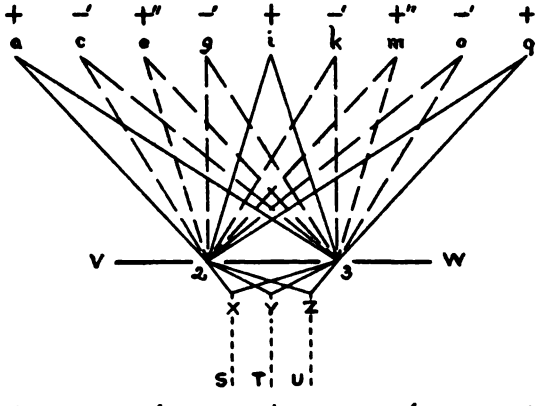


Fig. 8.

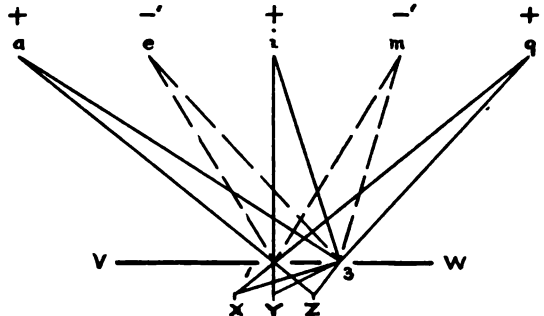
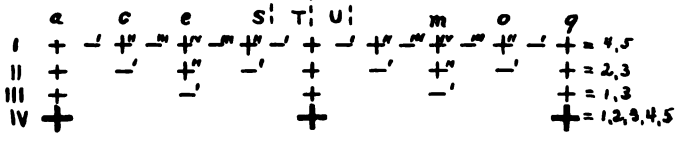


Fig. 9.



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both objectives and sub-stage condensers are commonly not aplanatic, different zones having different foci. The effect of projection by one zone of an objective may be seen with one focusing and that of projection by a different zone with another focusing. The importance of having but one focus for all zones of the objective [and of the sub-stage condenser which by having different foci for different zones sends primary rays, at any one focusing, only to the one zone in the objective corresponding to that which is at the time operative in the sub-stage condenser] becomes evident on studying Fig's 6, 7, 8 and 9, and corresponding photo-micrographs.

In Fig's 6, 7 and 8, let *VW* be a section of a diaphragm in contact with the emitting surface of a projecting lens. Let *X*, *Y* and *Z* be three self-luminous points (or points illuminated by a full cone of sub-stage light) in one of the conjugate foci of the projecting lens, and *a*, *i* and *q* primary ray images of *X*, *Y* and *Z* in the other of the conjugate foci. In Fig. 6, the diaphragm *VW* has two slots of which *4* and *5* are transverse sections; in Fig. 7, two slots of which *2* and *3* are transverse sections; and in Fig. 8, two slots of which *1* and *3* are transverse sections. The distance between the slots *4* and *5* is twice as great as the distance between the slots *2* and *3*, and four times as great as the distance between the slots *1* and *3*. The distance between *X* and *Y* (and *Y* and *Z*) is supposed to subtend a very small angle at the center of the projecting lens. [The distance is thousands of times the actual distance; and, as in previous diagrams, the wave length used is thousands of times too great. This is necessary to enable us to deal with measurements great enough to be useful.] Each slot is supposed to uncover one point of emission. Primary rays are indicated by unbroken lines, and secondary rays by broken lines. The broken character of the lines in any one of the diagrams increases with the obliquity of the secondary ray to its primary ray. For instance, *4k* and *4m* in Fig. 6 are secondary rays from the primary ray *4i*. The line *4m* is more oblique to *4i* than *4k* is to *4i*; and *4m* is more broken than *4k*.

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In Fig. 8, each slot allows one primary ray to reach each of the images *a*, *i* and *q*. The images *a*, *i* and *q* are not as perfect as the diagram indicates; for there would be to the right and to the left of the points *a*, *i* and *q* such spreading of light as we have before fully considered. At each slot in Fig. 8 a primary ray gives rise to secondary rays. One secondary ray from each slot reaches *e*, and one from each slot *m*. [In this, and Fig's 6 and 7, secondary rays to the left of *a* and to the right of *q* are not considered. They would take paths symmetrical with the secondary rays in paths to the right of *a* and to the left of *q*, respectively.] The secondary rays *1e* and *3e* differ in length by half a wave length, are opposed in phase and interfere; and *e* is dark. Correspondingly, *m* is dark. Over *a*, *e*, *i*, *m* and *q* in Fig. 8 are plus and minus signs indicating illumination and absence of illumination, corresponding to the same signs in Fig's 1 and 2.

After studying Fig's 1, 2 and 8, it is easily understood how the emitting points *2* and *3*, in Fig. 7 [twice as far apart as *1* and *3* in Fig. 8] project images of *X*, *Y* and *Z* at *a*, *i* and *q*, with diffraction patterns extending to the right and left of *a*, *i* and *q*, respectively, equal to half those projected under the conditions of Fig. 8. Thus *c* is dark, the result of union of secondary rays opposed in phase; while *e* is illuminated, the result of the union of secondary rays agreeing in phase. In a similar way, *g*, *k*, *m* and *o*, with their plus and minus signs, are accounted for.

In Fig. 6, the diffraction pattern, indicated by the plus and minus signs above the letters *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l*, *m*, *n*, *o*, *p* and *q* and projected by emitting points *4* and *5* [twice as far apart as the emitting points *2* and *3* in Fig. 7], is also easily accounted for in a manner similar to that in which we have accounted for previous diffraction patterns.

Now let us suppose all the emitting points, *4*, *2*, *1*, *3* and *5*, in Fig's 6, 7 and 8, to be simultaneously effective. The patterns of Fig's 6, 7 and 8 would unite. The result of the union is imperfectly expressed in Fig. 9, where the plus and minus values of the three diffraction patterns are brought

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together and added, as it were. Line *I* stands for the pattern of emitting points *4* and *5* in Fig. 6; line *II*, for the pattern of emitting points *2* and *3* in Fig. 7; line *III*, for the pattern of emitting points *1* and *3* in Fig. 8; and line *IV*, for the picture which union of the three patterns tends to produce (or the image of *X*, *Y* and *Z* the emitting points *4*, *2*, *1*, *3* and *5*, simultaneously effective, tend to project). The plus signs corresponding with the primary ray image *a*, *i* and *q*, reinforce one another, while the plus and minus signs corresponding to secondary ray interference phenomena tend to nullify one another. Fig. 9 [incomplete and not accurate*] serves to express graphically the general truth that multiplying the number of effective slots, or zones, intensifies the primary ray image, while diffraction phenomena tend to disappear.

Of the diffraction patterns projected in Fig's 6, 7 and 8, that of Fig. 8 [line *III* in Fig. 9] is nearest like that which the five slots jointly tend to project. But the latter is much more intense, and the spreading of light to the right and left of *a*, *i* and *q* is only one-fourth that in the former. The latter approaches four times as near being a dioptric image.

If *X*, *Y* and *Z* were sections of self-luminous lines (parallel with slots in the diaphragm *V W*), *a*, *i* and *q* would be sections of primary ray image bands. In addition to the bands *a*, *i* and *q* in Fig. 7 two diffraction lines, *e* and *m*, would appear. In Fig. 6 would appear six diffraction lines, *c*, *e*, *g*, *k*, *m* and *o*. The actual number of lines to the inch in the object would appear to be doubled, and half as far

* Some points not shown, or not shown accurately, in Fig. 9: The primary ray image projected by five slots has a brightness greater than that projected by any two of the same slots in the ratio of five to two [due to the actual increased amount of light leaving the lens and reaching the image]. Again, when the five slots are simultaneously effective the diffraction phenomena of line *I*, Fig. 9, can occur but once, because of the five slots only two are sufficiently far apart to project such a pattern; while the diffraction phenomena of lines *II* and *III* are intensified by more than one inter-pairing of the five slots. Therefore the latter patterns have more light, respectively, than the former. On the other hand, the diffraction phenomena of line *I* are contracted laterally [as by a wide aperture] so as to be twice as intense as the diffraction phenomena of line *II*, and four times as intense as those of line *III*. The intense thin diffraction phenomena of line *I* are likely to show under certain conditions as residua of opposing diffraction effects, even when the slots are made a continuous aperture by cutting away the intervening bars—as is shown at *c*, *e* and *g* (corresponding to the same letters in Fig. 6) in Photo. 29. Moreover, to Fig. 9 should be added another diffraction pattern projected as the effect of the inter-pairing of slots three times as far apart as slots *1* and *3*, which would present two equidistant plus phenomena between *i* and *q*, instead of the three shown in Fig. 6.

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apart, in Fig. 7; and quadrupled, and one quarter as far apart, in Fig. 6. If *V W* were a section of every diameter of the diaphragm, the slots *2* and *3*, and *4* and *5*, would become annular or zonular. Then *i* in Fig. 7 would be surrounded by a diffraction ring of light of which *em* would be the diameter. At *e* this ring would touch a similar ring surrounding *a*, and at *m* another similar ring surrounding *q* [dots and rings in the middle upper portion of Photo 6]. In Fig. 6, *i* would be surrounded by two rings having, respectively, the diameters *gk* and *em*. Two similar rings would surround *a* and *q*. The larger ring about *i* would touch the larger ring about *a* at *e*, and the larger ring about *q* at *m*. If *X*, *Y* and *Z* were sections of self-luminous lines, each point in a line would be projected as a disc with concentric rings. The discs would overlap in one direction to form a band, while the rings would cut one another so as to emphasize tangential lines parallel to the bands [see bands, semi-rings at their ends, and parallel tangential lines between them, in several portions of Photo. 6].

Experiment 5: The apparatus and its arrangement were the same as in Experiment 3, with two exceptions. The exceptions were: first, for the two pinholes were substituted three pinholes (in line, about ½ mm. in diameter and 2 mm. apart from center to center) and, near the pinholes, a group of three parallel slits (about ½ mm. wide and 2 mm. apart middle to middle); and, secondly, for the diaphragms with a single opening used at the back of the objective were substituted diaphragms with two slots. Images of the pinholes and slits were projected by the sub-stage condenser, with full aperture, in the plane of the microscope stage, where they became essentially self-luminous aërial dots and lines. The dots corresponded with the self-luminous points *X*, *Y* and *Z* in Fig's 6, 7 and 8. Diaphragms were made to correspond with *VW* in the diagrams. The distance corresponding to *1-3* in Fig. 8 was 1 mm.; the distance *2-3* in Fig. 7, 2 mm.; and the distance *4-5* in Fig. 6, 4 mm. A fourth diaphragm had five slots, 1 mm. apart, which would uncover five points of

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emission corresponding with *4*, *2*, *1*, *3* and *5* in the diagrams. The slots were equal in length.

The picture of the dots and lines seen through the microscope, with the full aperture, is shown in Photo. 1 as *a*, *i* and *q*, and at *a*, *i* and *q* repeated at an angle of 90°. [When taking the photomicrographs: an arc light and bull's-eye lens were substituted for the lamp; between the pinholes and slits, in a black card, and the sub-stage condenser was a blue light filter; the microscope tube was horizontal and the mirror was not used.] The full aperture image shown in Photo. 1 is nearest like the original object. Projected with five isolated slots, the image shown in Photo. 5 differs more; while with two isolated slots, the picture shown in Photo's 3 and 4 (corresponding with Fig's 7 and 6, respectively) differs remarkably from the original object.

Photo's 1, 2, 3, 4 and 5 present a pictorial warning against the use of isolated zones [each behaving like two slots, in every diameter], often utilized unwittingly by the practical worker [a result of imperfect correction of spherical aberration in either the objective or sub-stage condenser]; for if such simple details as we have studied are changed in appearance so remarkably, imagine what must be the corresponding changes presented to an observer when studying complicated structures!

In taking Photo. 1, an aperture of 10 mm. was used. In taking Photo. 29, a continuous aperture of about 4 mm. (just sufficiently wide to take in the emitting points *4* and *5* of Fig. 6) was used. In the latter a faint diffraction pattern, corresponding to that in Fig. 6, is seen partly at *c*, *e* and *g*, and partly between *i* and *q*; while in Photo. 1 such diffraction phenomena are absent. Great intensity of the light used, or long exposure of the photographic plate, may favor the projection of such a diffraction pattern as that seen in Photo. 29 [residual—see footnote, p. 351].

This matter of intensity of light in relation to aperture is worthy of little attention. If late in the day a microscope be arranged so as to resolve an object, with deepening twilight the closest and presently the less close details disappear.

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Similar phenomena are met with in ordinary work as the result of poor illumination. On the other hand, "drowning" with an excess of light [sometimes softened by using a blue glass filter] is an effect commonly seen.

There is an intermediate intensity best for microscopic vision. Bearing this in mind, if the intensity of the light in primary ray image bands were to measure twenty units and that of certain parallel diffraction lines one unit, the latter being barely visible, illumination could be lessened ninety-five per cent and still be sufficient to enable an observer to see the primary ray image bands, while the diffraction lines would be far too faint to be seen. On the other hand, if the diffraction lines of one unit intensity [barely visible] were to be illuminated twenty times as brilliantly to give them proper visual intensity, the primary ray image bands would be twenty times too intense and might suffer from "drowning" effects. The less the difference in intensity between the primary ray image bands and diffraction lines, the more likely are diffraction phenomena to obtrude themselves in a picture when the primary ray image has proper visual intensity. The use of pairs of slots or isolated zones, as compared with the use of continuous apertures, lessens this difference in intensity and favors the obtrusion of diffraction phenomena [compare *c*, *e* and *g* in Photo's 4 and 29, the former taken with a pair of slots and the latter with a continuous aperture].

Experiment 6: The general arrangement of the apparatus and the aërial object remained the same as in the last experiment. But instead of rectangular slots, annular or zonular slots were used at the back of the objective.

When an annular slot exposed emitting points in a circle 2 mm. in diameter, the image discs were surrounded by rings of light having radii corresponding to *ae*, *ie*, *im* and *qm* in Fig. 7, and shown in the middle upper portion of Photo. 6. When the annular slot exposed emitting points in a circle 4 mm. in diameter, the discs were surrounded by closer rings, the radii of the smallest corresponding with *ac*, *ig*, *ik* and

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qo, and the radii of the next larger with *ae*, *ie*, *im* and *qm*, in Fig. 6, and imperfectly shown in the middle upper portion of Photo. 7. The tangential union of diffraction rings between the bands [overlapping discs] results in intense lines with which, by comparison, the semi-rings at the ends of the bands appear faint [better seen in Photo. 6]. Portions of each ring are lost by interference in the intervals between the bands and the tangential lines. The annular slot or zone 2 mm. in diameter used in taking Photo. 6 was complete [a coverglass elsewhere opaque with India ink], while that 4 mm. in diameter used in taking Photo. 7 was broken [a stencil cut out of black paper]. Of the diffraction rings in the photomicrographs, those projected with the former zone are continuous lines, while those projected with the latter are broken —showing how sensitive the diffraction pattern is to modifications of aperture. The difference is imperfectly seen in the half-tone reproductions.

In considering the photomicrographs up to this point we have, for the most part, dealt with intensity in the one diameter corresponding to the widths of the lines photographed. Because the slots used had the same length, the intensity depending upon such lengths remained the same notwithstanding the changes experimentally made in the other diameter. We shall now see that this is an imperfect view of the matter. We have dwelt upon the facts that the diffraction pattern is contracted with every increase in diameter and that this is true in all diameters. We know that with circular lenses an area of diffracted light [a portion of a diffraction ring or line] varies in extent inversely with the square of its diameter or aperture. Therefore the light intensity of such an area would vary directly with the square of the aperture, provided the amount of light transmitted by the objective were to remain the same. But the amount of light transmitted by the objective increases with the square of its aperture. This means that, independently of the contraction of the diffraction pattern, an area of light in the projected image varies in intensity with the square of the

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aperture of the lens. Increase of aperture, then, adds to the intensity of the diffraction pattern in two ways. The increase of intensity gained in one way must be multiplied by that gained in the other, to get the total increase. The result may be expressed thus: *The intensity of the diffraction pattern varies with the square of the square of the aperture.*

Experiment 7: The apparatus and conditions remained the same as in the last experiment, excepting: for the object was substituted an aërial image of a tiny pinhole; and for two or more slots back of the objective single slots were substituted.

Photo. 8 is a double photomicrograph of the aërial object. *E* had the first exposure of fifteen seconds, with a slot 2 mm. wide. The aërial object was shifted very slightly to the right. *F* had an exposure of 1,215 seconds, with a slot 1 mm. wide. Exposed on one plate, the two images had the same treatment, in development, fixing and washing, and, therefore, could properly be compared as to their intensities. [The triple broadening of the disc, by the lesser aperture, at once attracts attention.] The exposures were so timed as to show the first diffraction ring in each case, provided the expression at the close of the preceding paragraph be true. [The square of the square of the aperture 1 is 1. The square of the square of the aperture 3 is (3 x 3) x (3 x 3), or 81. The exposures were as 1 to 81.] In the negative it was impossible to say that the two rings differed in intensity. Unfortunately the half-tone process has failed to reproduce the rings.

As a dioptric image would not be contracted by increase of aperture, its intensity would increase with simply the square of the aperture. Broad image areas, except at their boundaries, also increase in intensity with simply the square of the aperture. As such areas are made up of overlapping images of points, of which the object may be considered an aggregation, any increase of intensity due to contraction of the overlapping discs within the boundary bands is balanced by a decrease due to less overlapping.

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That boundary bands of image areas and images of isolated lines are affected alike by aperture is shown in Photo's 25 and 26. They were taken under the same conditions present in taking Photo. 1, excepting that, instead of full aperture, a rectangular slot measuring 2 mm. by 6 mm. was used back of the objective. In taking Photo. 25, the length of the slot was horizontal; in taking Photo. 26, vertical. Careful measurements show that there is broadening of the areas *A* and *B* with the lesser aperture in each case, and that the broadening is such as can be accounted for by the widening of the boundary bands. The narrower the area, the more important to the total breadth of the area is the broadening of the boundary bands. With small apertures the visual pictures of finest particles and lines consist chiefly of diffraction spreading. Such diffraction spreading not only contracts with increasing aperture, but gains in intensity rapidly [with the square of the square of the aperture]. Thus, increasing aperture narrows and intensifies most noticeably the picture of finest details. In a similar way, increasing aperture causes diffraction spreading of boundaries of areas to contract and to approach in intensity that of the area, as the intensity of the former increases with the square of the aperture, while that of the latter increases with the square of the square of the aperture.

Does not a study of Photo's 25 and 26 suggest a field for thought regarding micrometry? For instance, recall the disagreement of numerous careful workers who have measured blood corpuscles. Has one such worker recorded any consideration of the aperture used? Will not such a record come to be regarded as an essential part of critical micrometry?* While the percentage of error may not be great in

* A column might be added to numerical aperture tables to show what amount should be deducted for each N. A., with, for instance, an amplification of one hundred diameters. Astronomically, the diameter of, for instance, the sun's true image is increased at each end by half the diameter of a star disc; and, therefore, a measurement of the sun's diameter should be corrected for the aperture of the telescope used by subtracting the diameter of a star disc measured, if measurable, by means of the same instrument, or one of the same aperture. The uncertain [fading, not sharp] character of the visual boundaries of projected images also contributes to the uncertainty of micrometry.

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the measurement of broad areas, it is likely to increase rapidly as comparatively minuter objects and details are measured.

That a boundary of an area and an isolated line are affected alike by aperture is again shown in Photo's 23 and 24. The former, of an aërial image, was taken under the same conditions under which Photo. 1 was taken. The latter was taken under the same conditions under which Photo. 2 was taken. The right boundary is continuous with an isolated line, both of which have been affected alike in Photo. 24 by aperture. A study of the boundaries of the broader areas and of the dots and lines in Photo's 2, 3, 4 and 5, as compared with the same in Photo. 1, furnishes more evidence to the same effect.

A boundary band is not exclusively affected by aperture. Its next overlapping neighbor, and each succeeding band in the light area, has the same diffraction pattern. The lines of the diffraction patterns touch each other and produce the step-like areas of graded tints outside the boundaries of the light areas, seen in Photo. 5 [poorly shown in the reproduction]. Residua of these tints are also seen at the horizontal boundaries of *A* in Photo. 25 and at the vertical boundaries of *A* in Photo. 26.

The formation of these tints can be explained by means of Fig. 7. Suppose *YT* and *ZU* to be lines in a light area extending from *YT* to the right far beyond *ZU*, and *XS* to be absent. The area may be regarded as made up of lines parallel to *YT* touching one another. Images of the lines would be projected as overlapping bands extending from *i* to the left beyond *a*. Each successive band in the image to the left of *i* would, if isolated, have a diffraction pattern indicated by *k*, *m*, *o* and *q*. Thus *k*, *m*, *o* and *q* would be repeated for each successive band. To the right of the right boundary of the area projected to the left of *i*, the repetitions of *q* just touching one another would extend from *q* to *i*, and the repetitions of *m* from *m* to *i*. The tint between *q* and *m* would be, for the most part, an intensity of light resulting from the simultaneous effect [compounded wave motion] of the repetitions of plus *q* and minus *o*. The tint between *m* and *i*

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would be, for the most part, an intensity of light resulting from the simultaneous effect of the repetitions of plus *q*, minus *o*, plus *m* and minus *k*. Because the phenomena between *m* and *i* are more intense than those between *q* and *m*, in inverse relation to their obliquity, and because the phenomena between *q* and *m* would be repeated and compounded with the phenomena between *m* and *i* in the overlapping of diffraction patterns [the intensity of light between *q* and *i* being only slightly increased by the overlapping of fainter portions of the pattern which are not indicated in the figure but lie to the right of *q*], the tint between *q* and *m* would be considerably less bright than the tint between *m* and *i* [and the next tint to the right of *q* would be proportionately faint].

If the projected image of *YT* were fully shown by the pattern extending from *a* to *q*, repetitions of the pattern to the left of *a* would mutually and completely overlap one another, and brightness would there be uniform. Between *a* and *e*, within the image of the bright area, overlapping would be incomplete; and between *e* and *i*, also within the image of the bright area, less nearly complete. As a result: a tint, less bright than that of the image area to the left of *a*, would be seen between *a* and *e*; and a tint of still less brightness, between *e* and *i*. These tints within the image of the bright area would be more intense than the tints outside the image area, where overlapping becomes gradually less and finally does not occur.

We are now prepared to reverse our explanation and show how such boundary tints disappear when isolated slots are replaced by a continuous aperture. Because boundary tints depend upon the presence of diffraction patterns, the former disappear with the disappearance of the latter. The right boundary line of one tint is *m* [a false image corresponding to nothing in the object]. The repetition of this diffraction line between *m* and *i* essentially constitutes the tint in that situation. Then, in order to eliminate the tint between *m* and *i* from the picture, it is only necessary to eliminate the line *m* from the diffraction pattern.

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Suppose every point of emission between *2* and *3* in Fig. *7* to be uncovered, the point *1* [see Fig. 8] would emit light along a path to *m* half a wave length longer than the path *3m* [the path *2m* being in the diagram a wave length longer than *3m*]. A ray from each succeeding point between *2* and *1* would take a path to *m* half a wave longer than a ray from each succeeding corresponding point between *1* and *3* would take to *m*. The rays in the paths between *2m* and *1m* being longer than the rays between *1m* and *3m* by half a wave length, pair by pair, would be opposed in phase. The false line *m* would be replaced by darkness. Therefore darkness would extend from half way between *qo* on the right to half way between *ki* on the left. *SX* not being present *q* would be a first diffraction line, produced by the union of secondary rays in paths between *2q* and *1q* with those in paths between *1q* and *3q*—the former being, pair by pair, a wave length longer than the latter. If *SX* were present, the primary ray image at *q* would be intensified by the union with it of the same secondary rays. We thus see that all emitting points in an aperture are important. By using them, as compared with the use of isolated slots or zones, the picture improves by eliminating from it false diffraction appearances.

Photo. 30 is of aërial lines taken with the same general arrangement of apparatus. But for the sub-stage condenser another—a Powell and Lealand achromatic of ½-inch focus—was substituted; and for the objective, a Powell and Lealand ½-inch. At the back of the objective was placed a diaphragm having three slots corresponding to *4*, *1* and *5* in Fig's 6 and 8, or the equivalent of a pair of slots corresponding in distance apart to *4* and *5* in Fig. 6, or a pair and a half of slots corresponding in distance apart to *2* and *3* in Fig. *7*. The effect of such a combination is indicated in a general way by an imaginary union of the lines *I* and *II* of Fig. 9, and is seen in Photo. 30 at *a*, *e*, *i*, *m* and *q*. Plus *e* and plus *m* of the first line unite with plus *e* and plus *m* of the second, in Fig. 9, to form the bright lines *e* and *m* of Photo. 30. Owing to the contraction in the breadth of the plus effect represented

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by *c*, *g*, *k* and *o* in the first line, the effect is 100 per cent more intense than the minus effect of *c*, *g*, *k* and *o* in the second line when both are projected by two equal slots. But the latter effect now projected by three slots has its intensity increased by 50 per cent. This intensity, however, is still 50 per cent less than that of the former, projected by only two slots [only two of the three being far enough apart]. Residua of plus effect corresponding to *e*, *g*, *k* and *o* in the first line give the corresponding faint lines seen in Photo. 30 (but indistinctly shown in the half-tone reproduction between the lines *a* and *e*, *e* and *i*, *i* and *m*, and *m* and *q*). The same pattern is better shown in the reproduction of Photo. 31.

The different effects studied by means of Fig's 6, 7, 8 and 9, and shown in Photo's 2, 3, 4 and 5, depend upon varying the distances between the slots, the distance between *X* and *Y*, or *Y* and *Z*, remaining the same. The same effects may be got in the inverse way by varying the distances between the lines or details of an object, the distance between a given pair of slots remaining the same, as may be seen in Photo. 4 in which projection by two slots, corresponding with *4* and *5* in Fig. 6, causes the bands *a*, *i* and *q* of a primary ray image to appear four times too numerous to the inch. To the right of these bands is projected by the same slots a picture of lines half as far apart as the lines *a*, *i* and *q* [compare the two series of lines as shown in Photo. 1], which presents the same appearance an image of *a*, *i* and *q* would present if projected by two slots corresponding with *2* and *3* in Fig. 7.

In Photo's 29 and 30 are seen somewhat different effects due to varying distances between lines, the diaphragm back of the objective remaining the same. The image to the right of *a* in Photo. 30 was projected, as we have seen previously, by three slots corresponding to *4*, *1* and *5* in Fig's 6 and 8. The pattern just to the left of *a* is that which would be projected by slots corresponding to *4* and *3*, or *2* and *5*, in Fig's 6 and 7. Compare this with the corresponding, but residual, pattern under *1.5* in Photo. 29. The pattern *c*, *e* and *g* in Photo. 29 is similar to that which would be pro-

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jected by slots *4* and *5* in Fig. 6, or that shown in Photo. 4. The pattern under *1* in Photo. 29, with one faint diffraction line between the bands, is similar to that which would be projected by slots *2* and *3* in Fig. 7, or that shown in Photo. 3 at *e*, *m*, *a*, *i* and *q*. The pattern to the left of the last, barely showing resolution, is similar to that which would be projected by slots *1* and *3* in Fig. 8, or that shown in Photo. 2 at *a*, *i* and *q*.

Although Photo. 29 shows patterns similar to those which a pair of slots would project of lines different distances apart, the picture was in reality taken by a continuous aperture—a slot just wide enough to barely resolve the closest series of lines shown as image bands in the first position [above and to the left in Photo. 29], twice wide enough to resolve the series shown in the second position, three times wide enough to resolve the series shown in the third position, and four times wide enough to resolve the series shown in the fourth position. Between *a*, *i* and *q* in the fourth position are seen the residua already mentioned corresponding to *c*, *e* and *g* of Fig. 6 or of Photo. 4. Between the image bands in the third position are seen the residua of the corresponding broader lines of Photo. 30. Between the image bands in the second position are seen the residua of lines corresponding to *e* and *m* of Fig. 7 or of Photo. 3.

Why do residua of light corresponding to diffraction lines show in Photo. 29 and not in Photo 1 ? Partly because Photo. 29 had a relatively long exposure, Photo. 1 having only sufficient exposure to enable the primary ray image to properly affect the sensitized plate. The sensitized plate saw, as it were, the projected image in Photo. 1 with a proper intensity of light. In Photo. 29 the intensity was unnecessarily great, but not great enough to give the "drowning" effect seen in Photo's 6 and 7. If Photo. 1 had received relatively the same exposure given Photo. 29, the greater aperture effective in taking the former would have contracted the pattern so that the diffraction lines could not have been seen as separate from the primary ray bands without the

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aid of a pocket lens. This was found to be true by actual test.

To what is the "drowning" effect seen in Photo's 6 and 7 due? In the beginning of this paper the inverse relation of the sizes of image discs, due to uncorrected spherical and chromatic aberrations on the one hand and to aperture on the other, was noted. In so-called achromatic objectives these aberrations are at best incompletely corrected. In apochromatics they are not perfectly eliminated. Residual aberrations increase with aperture. Aberrant light, if isolated, would give rise to aperture diffraction phenomena. It would be reflected in ways similar to those we have studied in the instance of primary ray light. The rays of each color would project patterns peculiar to and depending upon their respective wave lengths. Not isolated, aberrant rays unite at lens surfaces with other rays leaving the same points, to be compounded in resultant wave motion. Thus, aberrant light complicates wave motion. This results in a failure on the part of the proper proportion of rays to fully nullify one another and in residual diffraction phenomena spreading out in the picture beyond the boundaries and in the interstices of the primary ray image. Sufficiently brilliant illumination gives such residual light a visible intensity. Aberrant reflections at numerous lens surfaces, any method of illuminating an object which brings a zone of aperture into separate use, and certain accidents of construction in the objective or substage condenser, result in an overspreading of light the intensity of which increases with the brilliancy of illumination. As a result of one or more of these several causes the intervening spaces between the details of microscopic images come to be occupied by more or less light. Under conditions of ordinary usage it is not particularly noticeable. But by excessive brilliancy of illumination or by excessive aperture (relatively to poor correction of aberrations) increasing residual aberrations, or by both, it may become visible or with prolonged exposure affect the photographic plate. This is evident in the results got in several negatives which were

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secured in attempting to photograph diffraction rings, especially those of second and third intensity shown in Photo. 7. [Such an explanation of "drowning" effect suggests an unrecognized factor—so far as the writer knows—in the production of photographers' "halation" which has been thought to be due wholly to reflection from the back surface of the negative plate or to the lateral spreading of chemical action in the film, or both. On seeing the "drowning" effects in Photo's 6 and 7, the photographer would say they were due to "halation."]

It was found that a thin cell with plate glass sides containing a deep blue solution of ammonio-sulphate of copper placed anywhere back of the sub-stage condenser rids a visual picture of much of the "drowning" effect by reducing the intensity of illumination and eliminating some of the phenomena due to other than blue wave lengths. Even with the blue cell, which is less monochromatic with the increasing intensity of the light it filters, long exposure gave the results seen in Photo's 6 and 7 [on double-coated "non-halation" plates which gave better results than the ordinary plates also tried].

Experiment 8: The apparatus was arranged as it was when Photo's 1 to 6 were taken. But instead of an aërial image of bright lines and areas an aërial image of dark lines and areas was photographed. The results in Photo's 19, 20, 21 and 22 correspond with those in Photo's 1, 2, 3 and 5, respectively. Broadening of black bands in the image by narrow aperture is seen in Photo. 20; the production of false diffraction lines (*e* and *m*), in Photo. 21; and the effect of using five slots instead of two, in Photo. 22. In this experiment the results are apparently analogous to those obtained with bright lines as an object. But the black details are fainter than the bright details in the corresponding photomicrographs. This was more marked before Photo's 19, 20, 21 and 22 were intensified. [These alone of the photomicrographs submitted were intensified to aid the half-tone plate makers.] On comparing broad areas the positive or bright effects tend to

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overbalance the negative effects. Bright areas in a dark field appear too broad, due to spreading of light into the dark field. Dark areas in a bright field appear too narrow, due to spreading of light from the field into the dark areas. This is seen in comparing *A* and *A* in Photo's 1 and 2 with *A* and *A* in Photo's 19 and 20.

Photo. 23 is of an aërial image taken under the same conditions under which Photo's 1 and 19 were taken; and Photo. 24, of the same aërial image taken under the conditions under which Photo's 2 and 20 were taken. In the upper part of the picture is seen a negative band (*a*) in a bright area and also a bright band (*b*) in a negative area. In Photo. 24 the false diffraction line duplicates of these two bands are formed as though the fellow band and duplicates were absent. The diffraction duplicates and the primary ray image bands vie with each other for the same places in the picture, with a result to the advantage of the bright line at every place. [The half-tone reproductions show the negative effects imperfectly—hardly at all.]

Perhaps the best way to study the two series of photomicrographs compared above, and Photo's 23 and 24, is to attend chiefly to the bright lines and areas: to consider the positive effects of aperture in relation to the bright boundaries of bright areas, the bright boundaries of fields surrounding dark areas, the bright bands and bright spaces between dark bands; and, on the other hand, to regard the dark areas and bands as negative interruptions having only corresponding interrupting effects in the projected image. "Drowning" effect would tend to weaken the intensity of the image of dark lines and particles, and to narrow images of broad areas of black. In critical micrometry it may be necessary not only to have a regard to aperture, but also as to the positive or negative character of the picture.

Experiment 9: The general arrangement of the apparatus remained unchanged. An aërial image of the lines of Fig. 4 was projected by means of a Powell and Lealand achromatic sub-stage condenser of $\frac{1}{3}$-inch focus, using dia-

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phragm opening "3." The resulting aërial image was photographed with a Powell and Lealand ½-inch objective and 2-inch eye-piece to get Photo. 27. Photo. 28 was then taken after making but one change in the arrangement. Diaphragm opening "3" of the condenser was changed for the smallest, "1." Resolution fell off proportionately. Several diffraction lines may be seen in Photo's 27 and 28. Photomicrographs might be produced to show that diffraction phenomena parallel with those we have already studied occur in images projected by the sub-stage condenser.

Early in this study a telescope lens was used as the objective of a gigantic microscope. In the present experiment a microscope objective [for the sub-stage condenser is essentially such] was used as that of a telescope. The two instruments behaved alike. Moreover when a transparent object resting upon the microscope stage is illuminated by an image of the source of light projected in the plane of the object, it appears that diffracted light may be a factor of illumination and that a change in openings of the sub-stage diaphragm may alter the character as well as the intensity of illumination.

[Compare, for a moment, Photo's 27 and 28 with Fig. 4, and note the difference between the original object, as shown in Fig. 4, and the trebly-projected images. What a falling off is to be seen in the final pictures! a result of imperfection in lens projection. As we have double projection in ordinary practice with the microscope, we may believe microscopic vision has nearly two-thirds of the imperfection seen in Photo's 27 and 28 as compared with Fig. 4 when correspondingly high-power eye-pieces are used.]

Experiment 10: A telescope was arranged as in Experiment 2. Instead of the paper diaphragm with a single slot, diaphragms with two or more slots were used in the hood. Emitting points uncovered by pairs of slots (or an isolated zone of aperture) in a telescope behaved as we have seen the corresponding apertures of a microscope objective behave in the projection of diffracted phenomena.

Experiment 11: Fine and closely-ruled lines were

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observed while diaphragms with minute openings were held between the lines and the eye. The conditions were varied so as to convince one that the dioptric apparatus of the eye projects diffraction phenomena parallel with those previously studied in images projected by the telescope, microscope and camera objectives.

It is a matter of common observation that black articles look smaller than white articles of the same size. A filament of black carbon appears broader when incandescent in the electric lamp. The eye is not corrected for spherical and chromatic aberrations. These aberrations cause light areas to appear to encroach on dark ones. Our experimental study suggests that diffraction effects are also a factor in the phenomenon. The apparent spreading of light into darkness increases with the intensity of the light. The brighter a star, the larger it looks. Is not this phenomenon, "irradiation," to the retina essentially what "halation" is to the sensitized film of the photographic plate?

The optical imperfections of the eye and the effects of its aperture, the pupil, contribute with those of the instruments we have considered to project on the retina images differing to a varying degree from the original object.

That the effect of aperture is important in the eye-piece is shown by the influence variation in the size of the opening in the eye-piece cap has on resolving power, both photomicrographically and visually. The field-lens is functionally a part of the objective, the two projecting the image at the diaphragm between the field-lens and eye-lens. The eye-lens and the lens of the observer's eye are a projecting combination capable of projecting on the retina all the diffraction phenomena we have found other lenses project.

Experiment 12: Photo. 32 shows the lines of an Abbe test plate [the same shown, inverted, in Photo's 9 and 10, and described at page 337] taken when the last emitting surface of the objective used was covered with a diaphragm which had an eccentric opening 1 mm. wide and transmitted only such rays as had been previously diffracted in the plane

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of the object by the lines cut through a film of silver. Photo. 33 was taken under the same conditions, excepting that the eccentric opening was 2 mm. wide. The effect of increase of aperture, narrowing lines and favoring resolution, when using only such rays as had been diffracted by the object may be seen in the indistinct resolution in the upper half of Photo. 33. This is more distinct in the photomicrograph. Photo. 34 was taken under the same conditions as Photo. 32, excepting that the slot 1 mm. wide transmitted central primary rays. Photo. 35 was taken with a slot 2 mm. wide, half central and half eccentric, transmitting through its central half primary rays and through its eccentric half diffracted rays. Since the half-tone reproductions illustrating this comparative experiment were made, the experiment itself has been repeated (visually) several times. As a result the writer finds that when Photo. 33 was taken the width of the slot was not wholly filled with diffracted rays. This means that the *effective* aperture was a little less than 2 mm. wide. *Aperture beyond that which is utilized, or effective, contributes nothing to resolving power.* An accidentally lessened aperture is thus shown to be responsible for the inferior resolution in Photo. 33 as compared with that in Photo. 35. Repetitions of the experiment show that under parallel conditions the same aperture gives the same resolution with either diffracted or primary rays. In other words: *Aperture affects diffracted rays from an object as it does primary rays from an object.*

Disregarding the accidental difference in resolution Photo's 32 and 33 differ notably from Photo's 34 and 35. Certain negative imperfections, as breaks in a line, seen in the lower pair are also to be seen in the upper, the pairs being alike in this respect. Certain positive faults—scattered small splashes of light, images of little irregular areas broken through the silver film—seen in the lower pair are not to be seen in the upper pair or are replaced in the upper pair by corresponding negative faults. The positive faults in Photo's 34 and 35 were projected by primary rays. No primary rays contributed to the formation of the images seen in Photo's 32

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and 33, but only diffracted rays of limited range as to obliquity. The position of the eccentric opening in the diaphragm determined the necessary obliquity of the rays which could be transmitted. The closeness of the lines in the silver film determined the obliquity of the diffracted rays which reinforced one another [diffracted rays of other obliquities leaving the lines in the silver film nullified one another by interference]. The position of the eccentric opening in the diaphragm was such as to transmit rays of the obliquity determined by the lines in the silver film. Certain positive faults of various breadths in the silver film gave rise to diffracted rays which reinforced one another at various obliquities outside the grasp of the eccentric opening in the diaphragm. Such rays could not reach the picture. Their absence resulted in negative "splashes" in Photo's 32 and 33 corresponding with certain positive "splashes" in Photo's 34 and 35.

There are objects which may be satisfactorily illuminated with a spot-lens or a sub-stage paraboloid—or by oblique light obtained in any way—so that no direct rays reach the objective, but only those changed in direction as by refraction or diffraction. Under such circumstances and in the case of a uniformly fine structure, for instance, the cone of rays taking paths to the objective might be exclusively diffracted light. Thus the image would be projected wholly by diffracted rays which on passing through the instrument would be affected by aperture as we have seen primary rays are affected by aperture. There would be one peculiarity about the intensity of such light in the cone between the object and the objective. The diffracted rays of greater intensity would be nearer the periphery; because the primary rays, from which they would originate, would be outside the cone. The rays would lose intensity with their nearness to the axis. [Diffracted rays originating from axial rays, as has been true in all previous instances cited, lose intensity in the opposite direction, or with their obliquity to the axis. This difference in the two kinds of cones may be shown to have some perceptible

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influence in the projected image.] The possibility of such illumination is confined to the exhibition of objects observed under low power objectives and belongs rather to the æsthetic than to the practical domain of microscopy.

If we consider opaque objects illuminated for microscopical observation, all the rays between the object and objective are indirect—by reflection. These indirect rays can travel only in the paths already considered and can be affected in no new way by aperture. Has it not become evident that the diffraction of light by an object may be considered in the same category with other changes in direction in incident light produced by an object, as, for instance, those resulting from reflection?

In taking Photo. 10, between the object and objective was used a cone which consisted of a full cone of primary rays and a full cone of diffracted rays uniting at the origin of the latter in the object to form a compounded resultant full cone. In the case of a similar but coarser object, illuminated by transmitted light, the primary rays might be confined [first] to peripheral paths, or [second] to axial paths in such a cone. In the [first] instance: there would be a full cone of diffracted rays originated by the lines of the coarser object which, if alone, would be affected in no new way by aperture; and in addition, primary rays uniting with the peripheral diffracted rays. If the apertures at lens surfaces were not to be operative later, the only final result, in a projected image, of the increased intensity in the periphery of the cone would be a greater brilliancy of the picture. But both the faint axial and the more intense peripheral rays are affected by aperture, the latter rays tending to produce the phenomena we have seen result from projection by a zonular aperture. If the fainter axial rays were to have the same focus, they would tend to correct the false diffraction effects [obtruding zonular aperture effects] in the picture, by giving origin at the emitting surface of the objective to secondary rays, from secondary rays, tending to complete the diffraction effects of a continuous full aperture. In

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the [second] instance: there would be the full cone of diffracted rays; and in addition, primary rays uniting with the axial diffracted rays. If the apertures at lens surfaces were not to be operative later, the only final result in the projected image of the increased intensity in the axial rays would be a greater brilliancy of the picture. But both the faint peripheral and the more intense axial rays are affected by aperture, the latter rays tending to produce the effects we have seen result from projection by a small circular aperture. If the fainter zonular rays were to have the same focus, they would tend to correct the defects [obtruding lesser aperture effects] in the picture, by giving origin at the emitting surface of the objective to secondary rays, from secondary rays, tending to complete the diffraction effects of a continuous full aperture.

In practice it has been found that when the general conditions of the [second] instance are made more specific,. *when primary rays utilize a central circular surface of emission having a diameter equal to about three-fourths that of the full aperture, conditions are favorable for best microscopic vision*, more favorable than when the whole of the last surface is utilized by primary rays. Increased spherical aberration due to increased utilization of aperture has been supposed to explain why a full cone of primary rays does not give so perfect a picture as the three-fourths cone. Mr. E. M. Nelson* has shown that increased spherical aberration can only in part explain the difference. A fuller explanation for this at first sight contrary effect of a smaller aperture is needed, and may be given, as follows:

When the horizontal width of a 5 mm. square aperture resolved lines, as shown in Photo. 13, variation in the height of the aperture was found to have no influence on resolving power. A photomicrograph of the same aërial lines was taken with an aperture height of 2 mm.; and another, with a height of 8 mm. [the horizontal width remaining 5 mm].

* *The Journal of the Quekett Microscopical Club*, March, 1895, p. 30.

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These photomicrographs only duplicated the result seen in Photo. 13.

Let us examine circular and square apertures in relation to the slots *4*, *2*, *1*, *3* and *5* of Fig's 6, 7 and 8. In Fig. 10 the slots *4*, *2*, *1*, *3* and *5* are shown bounded by full lines. The diameter of the circle *EFH* is three-fourths that of the outer circle *ABCD*. Let the area within the circle *ABCD* represent the last emitting surface of an objective. Let the object be a series of fine lines parallel with the length of the slots. Let *mnst* be a square aperture having a width equal to the diameter of the full circular aperture of the objective. The resolving power of a square aperture is about ten per cent greater than that of a circular aperture of the same width or diameter. The resolving power of the rectangle *opqr* is equal to that of the square, so far as the horizontal diameter of the objective is concerned [and this is the diameter upon which depends the resolution of the series of lines constituting our present object]. The other thirds of the square, the rectangular apertures *mnop* and *qrst*, have the same resolving power.

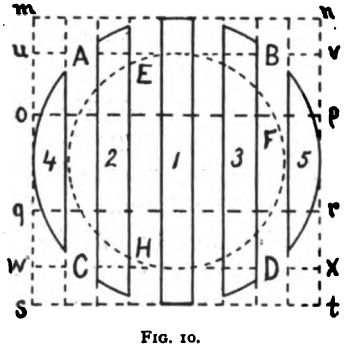

FIG. 10.

Now compare the portions of the slots within the middle rectangular aperture *opqr* with those within nearly the same area bounded by *op* above, *qr* below, and on the right and the left by the boundaries of the full circular aperture. The portions of slots *2*, *1* and *3* are the same. Those of slots *4* and *5* are nearly the same. If the upper and lower boundaries of the two areas were nearer, the portions of the slots *4* and *5* included in the area would be still nearer alike. If the upper and lower boundaries of the two areas were farther apart, the portions of the slots *4* and *5* included in the areas would be less alike. It becomes evident, then, that the portions of the circular and rectangular apertures between the lines *op*

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and *qr* have nearly the same resolving power, and that, if the lines *op* and *qr* were somewhat nearer, the included portions of the two apertures would have practically the same resolving power.

Compare in a similar way the portions of the slots in the upper third of the square with the portions of the slots the corresponding part of the full circular aperture would utilize. The corresponding part of the full circular aperture would utilize almost the same portions of the slots *2*, *1* and *3* that are in the upper third of the square, but only comparatively small parts of the portions of the slots *4* and *5* that are in the same third of the square. What is true in this respect of the upper parts of the square and the larger circle is also true of the lower parts of the square and larger circle between the lines *qr* and *st*. The diffraction effects of the upper part of a full circular aperture are reinforced by the same effects of the lower part. These united effects tend to be those of an aperture corresponding to *2*, *1* and *3* rather than of an aperture corresponding to *4*, *2*, *1*, *3* and *5*. Then portions, as to vertical diameter, of the circular aperture tend to produce the effects of a continuous aperture between and including the slots *2* and *3* in Fig. *7* ; and another portion, the effects of a continuous aperture between and including the slots *4* and *5* in Fig. 6. The result is intermediate aperture effects. In other words, a study of Fig. 10 gives us an explanation of the superiority in resolving power of a rectangular as compared with a circular aperture, a superiority we have already experimentally found to be about ten per cent.

Now, let the full cone of primary rays between the object and the objective be reduced one-fourth, to a three-fourths cone. The primary rays would then reach and fill the area within the circle *EFH*. Slots *4* and *5* would transmit no primary rays. Their diffraction effects in the image would be lost were it not for the fact that they would transmit diffracted light, diffracted by the object. The lines of the object being vertical, the diffracted light originated by them would be spread out horizontally to the left and the right, not vertically,

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and would fill the horizontally opposite portions of the zone lying between the circles *EFH* and *ABCD*. The portions of the slots *2*, *1* and *3* above the line *uv* and below the line *wx* would not be utilized. The relative importance of slots *4* and *5* would be increased, by thus limiting the effect of aperture to the area between the line *uv* above and the line *wx* below. As a result, resolving power would be correspondingly increased.

Were the cone between an object and the objective to be so reduced that primary rays would reach and fill only a circular emitting surface having a diameter one-third of that of full aperture, the effective aperture would be limited above by the line *op* and below by the line *qr*. It would be approximately the equivalent of the rectangular aperture in relation to the horizontal resolution of the vertical lines. The same or more brilliant resolving power might be got by leaving the cone between the object and objective full and using behind the objective a diaphragm with a rectangular opening corresponding with *opqr*. By narrowing sufficiently the vertical width of the rectangular opening the resolving power might be made equal to that of the square aperture. This is not peculiar to the microscope objective. It is equally true of the telescope objective.* It is possible, however, for the accompanying loss of light from, for instance, faint double stars, or from faint details of microscopic objects, to more than counterbalance this increase of resolving power, visually [long exposure photographically here having an advantage].

With a narrow rectangular aperture resolution effected by the length of the aperture is limited to one diameter of an object. When, on the other hand, the cone between an

* It would be advantageous for some purposes, if practicable, to construct a narrow and rectangular astronomical telescope objective. Such a lens would possess nearly ten per cent more resolving power than a circular lens having a diameter equal to the length of the narrow lens, would be comparatively light in weight and require less strength of support. Or, with the same extent of surface as the circular lens and about the same amount of glass and weight, the length of the rectangular lens might be increased so as to transmit as much light and have several times the resolving power. The position of such a lens revolved about its principal axis to get best resolution would indicate the direction of a line uniting the components of a double star. In instances when the intensity of the light is unimportant, the surface of such a lens might be cylindrical instead of spherical.

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object and the objective is reduced, the last emitting surface of the objective remaining uncovered, vertical lines have *a selective power*, as we have seen, utilizing horizontal aperture. In a similar way horizontal lines utilize a vertical aperture. Thus, any lines or details utilize appropriate aperture. "Selective power," if we may so call it, acts simultaneously and independently in different diameters, with a possibility of exhibiting details generally in an object ten per cent closer than the whole aperture filled with primary rays can exhibit. This is not peculiar to the microscope objective. It is equally true of any other lens projecting an image under parallel conditions.

As the Abbe theory requires a greatly reduced cone [for without such a cone diffraction by the object is nullified and "spectra" are absent], and as we have seen that a sufficiently narrow cone may so utilize the aperture of an objective as to make it the equivalent of a rectangular aperture in resolving power, we can account not only for the greater resolution of a three-fourths cone compared with that of a full cone, but for an excess of resolving power found in the Abbe numerical aperture tables.

Mr. Nelson has studied four kinds of illumination in practical work and finds the actual resolving power to be in the following order, the strongest first:

"Appearance at Back of Objective.

1. Peripheral annulus bright, $\frac{3}{4}$ center dark.
2. Peripheral annulus dark, $\frac{3}{4}$ center bright.
3. The whole dark (dark ground).
4. The whole bright (full cone)." *

Mr. Nelson finds this result not quite in harmony with theory. The difficulty may be explained by means of Fig. 10. The "peripheral annulus bright" is shown as the zone between the circles *ABCD* and *EFH*. In this zone are slots *4* and *5* and small portions of slots *2*, *1* and *3*. Because the preponderance of effective aperture corresponds with slots *4* and *5*, the resolving power of the circular aperture in this

* *The Journal of the Quekett Microscopical Club*, March, 1895, p. 34.

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instance approaches nearly that of a corresponding rectangular aperture. The "peripheral annulus dark, ¾ center bright," has already been shown to have a resolving power greater than that of the full circular aperture and less than that of a corresponding rectangular aperture. Its resolving power is less than that of No. 1 and more than that of No. 4 of Mr. Nelson's list. On first thought, one would expect No's 3 and 4 to be equal in resolving power. We have already noted [page 369] a peculiarity of a cone of light leaving the object under the conditions of illumination present in No. 3, *i. e.* it is more intense at the periphery. This means that the effects of a peripheral zonular aperture are somewhat developed in No. 3. In No. 4 illumination is uniform or less intense at the periphery. So No. 3 is the equivalent of No. 4 plus a little of the effects of No. 1. Mr. Nelson's difficulty may be met thus without reference to the Abbe theory.

Great reduction in the cone of primary rays between an object and the objective in an attempt to make a round aperture approximate in resolving power a rectangular aperture is not advisable. The projected image loses dioptric intensity, by the loss of primary rays, in proportion to such reduction. Diffraction phenomena become more evident in the picture. False appearances may obtrude themselves in the image as a result of the rays diffracted by the object utilizing isolated zones of the objective, especially when the isolated zones of the objective have corresponding isolated foci.

Moreover, depth of focus—slight with a full cone of primary rays—increases with reduction in the cone. A full cone primary ray image is so nearly limited to a plane that it comes into or goes out of focus immediately—not gradually, while a narrow cone primary ray image responds to focusing so gradually that it is difficult to say when focusing is correct. With a full cone of primary rays of proper intensity we have seen that diffraction patterns are contracted to a minimum and may be visually absent. On the other hand, with a narrow axial cone of primary rays from such an object as our last, dif-

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fraction patterns are broadened and multiple slot or zonular diffraction effects—due to the diffracted light from the object utilizing portions of the objective isolated from that utilized by the axial pencil—become pronounced in the image. The diffraction pattern may add force to a correct picture, or it may add false appearances such as we have studied in connection with Fig's 6, 7, 8 and 9, and shown in Photo's 2, 3, 4 and 5. It was noted that the diffraction pattern projected in Fig. 1 would appear on the screen if the screen were nearer or farther away from the sources of light. This is equally true of zonular diffraction patterns. Above and below the correct image plane are planes in which the zonular patterns may be focused. This phenomenon with the uncertainty of focusing correctly explains why an observer using too narrow a cone of light in illuminating an object is likely to see—by focusing isolated diffraction effects in other planes than that in which the primary ray is projected—diffraction "ghosts," and not the correct image at all.

Arrange such an object as closely-ruled fine lines for microscopic vision, illuminating it with a full or three-fourths cone of light from a sub-stage condenser. The bands of the primary ray image, all that can be seen, come into and go out of focus immediately, without gradually appearing or lingering. Now reduce illumination to a small axial cone. It becomes difficult to determine when the primary ray image is in focus. After the primary ray image is in focus, focusing upward or downward causes the picture to give place to imperfect repetitions of itself. Six or seven such repetitions, each succeeding one becoming more unlike the primary ray image, may be seen by focusing upward or downward. Such repetitions are diffraction effects, "ghosts" of the primary ray image; and while one "ghost" is changing into another more complicated diffraction patterns may be seen.

Theoretically and practically, then, we have seen that *advantageous reduction in a cone of light between an object and the objective may be made only within a limit. Beyond such a limit counterbalancing evils are likely to be met. The advis-*

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able reduction in the case of a first-class objective is found to be from about one-fourth to one-third, never more than one-half, of the diameter of the cone.* The diameter and angle of the cone of light may be roughly determined and varied by means of a sub-stage mirror, or critically determined and varied by means of a sub-stage condenser of high quality, *i. e.* made with the same care given to the making of the best objective. *If so much depends upon the cone of light between an object and the objective, and this cone may be critically controlled only by means of a sub-stage condenser of high quality, is not one impressed with the advisability of habitually using a first-class aplanatic sub-stage condenser, and using it intelligently?*

Experiment 13: The general arrangement of the apparatus was the same as that used in taking Photo's 1 and 19. An opaque card, having a cross-shaped hole cut through it, was placed against the bull's eye condenser—instead of the card and holes used in taking Photo 1. An aërial image of the cross was projected in the plane of the microscope stage by a 1-inch objective, arranged as a sub-stage condenser. This aërial image was then observed through a 1½-inch objective and a 2-inch Huyghenian eye-piece. Let Fig. 11 indicate diagramatically the relative positions of the sub-stage condenser, aërial image and objective. The dotted line *a* shows the position of the first lens of the sub-stage condenser, and *b* the position of the second lens of the sub-stage condenser. Let *c* show the position of the first lens of the objective, and *d* the position of the second or final lens of the objective. Let *4* represent the Powell and Lealand sub-stage diaphragm with circular opening "4" in use. Let

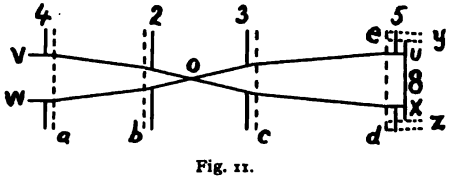

Fig. 11.

*See the description of Fig. 2 in the frontispiece of the Dallinger-Carpenter edition of THE MICROSCOPE AND ITS REVELATIONS.

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the angular lines *V O X* and *W O U* indicate the paths through the lenses which the boundary rays of the light transmitted by the diaphragm opening at *4* traveled. Experimentally it was found that a circular opening 2 mm. in diameter in a diaphragm capping the sub-stage condenser at *2*, a circular opening 3 mm. in diameter in a diaphragm capping the objective at *3*, and a circular opening 5 mm. in diameter in a diaphragm placed back of the objective at *5* just permitted all the rays transmitted by the diaphragm opening "4" to reach the emitting surface at *d*.

Then, without otherwise changing the arrangement, for diaphragm *5* with its opening 5 mm. in diameter was substituted diaphragm *8* [shown just back of *5*] which had a zonular opening and an opaque central portion 8 mm. in diameter. Diaphragm *8* obstructed all the primary rays emitted by the lens at *d*. The eye-piece was then removed. On looking at the back of the objective the zone *ed* uncovered by diaphragm *8* was illuminated and remained illuminated even when the circular opening at *4* was changed to "1" of the sub-stage diaphragm. The peripheral zone at the back of the objective was illuminated by rays which must have been separated from the direct axial rays at a previously operative lens surface.

The 2-inch eye-piece was replaced. The eye-lens of the eye-piece was removed. A 1-inch objective was mounted in the front board of an ordinary camera with the axis of the objective coincident with that of the microscope tube. The position of the camera with the objective was adjusted so as to project an image of the cross on the ground-glass of the camera with diaphragm *5* of Fig. 11 in place. The ground-glass was removed. In its stead was exposed a sensitized plate. The resulting photomicrograph is reproduced in Photo. 14.

Diaphragm *5* was then exchanged for diaphragm *8*. The field-lens of the eye-piece projected an aërial image of the annulus of light uncovered by the diaphragm *8*. This aërial image focused and photographed with the camera and its 1-inch objective is shown in Photo. 17. Immediately after-

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ward, while observing the image of the annulus on the ground-glass as the camera with its 1-inch objective was pushed slowly towards the microscope, the annulus was seen to shrink gradually and become the small cross shown in Photo. 18, which is inverted as to the larger cross in Photo. 14. There can be no doubt, then, that the annulus was illuminated by light which in some way was derived from the aërial image of the cross projected in the plane of the stage, light which must have left the direct axial rays at some point in their paths. The inversion of the smaller image shows that the eccentric rays while traveling paths through the lenses were converged to at least one more focus than the direct axial rays. Such additional convergence was necessarily the result of at least two lens surfaces acting twice, each acting as a reflecting as well as a refracting surface. The exact paths taken by the eccentric rays have not been traced. It is probable, however, that they were separated from the direct axial pencil by internal and converging reflection at the emitting surface of the front lens of the objective and returned to the front surface, thence to be reflected back in an outward direction to and through the emitting surface along eccentric paths toward the annulus. Of what moment, separately or as a factor in compounded wave motion, the utilization of aperture by such faint aberrant rays is in microscopic vision does not here concern us. The writer shows that such rays are present chiefly because the same and similar rays have been regarded as factors in phenomena previously considered in our study [page 331 and page 363].

The Abbe "spectra" now invite our attention. We have found that they are not indispensable in the projection of some of the images of microscopic vision. "Spectra" are images of an opening in the diaphragm of a sub-stage condenser, or of a source of light, formed above the objective in the microscopic tube by diffracted rays originating in the object. Can an image of an opening, or of a source of light, formed in the microscope tube between the objective and the field-lens of the eye-piece by diffracted rays have any influ-

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ence on the primary ray image of microscopic vision formed between the two lenses of the eye-piece? Two widely separated images cannot be seen through the microscope at the same time. In other words, such images cannot be united to form a joint visual picture.

The plane in which the Abbe "spectra" are seen, when present, is indicated by the dotted line *FF* in Fig. 2. The position of one of these "spectra" is indicated at the point in *FF* where the diffracted rays, dotted lines *vi* and *we*, cross. The rays of light in the paths *vi* and *we* leave the same point in the opening in the diaphragm of the sub-stage condenser as part of a primary ray pencil, diverge to the lenses of the sub-stage condenser, and are parallel on passing from the sub-stage condenser to the object *O*. As diffracted rays, *a' v* and *d'' w*, they travel parallel paths to the lenses of the objective. From the lenses of the objective they converge to a focus in a plane *FF*. As to the object [now *a'd''*] the two diffracted rays leave its opposite extremities. As to the image of the object [now *i e*] they go to its opposite extremities. Of the innumerable rays which leave one point in the opening in the diaphragm and reach a corresponding point in the image of the opening in the plane *FF* no two pass through a common point in the object or arrive at a corresponding common point in the image of the object. On the other hand, each of the rays *v i* and *w e* is but one of countless rays which leave a common point in the object and reach a corresponding common point in the image on the screen. Then the image in the plane *FF* of an opening in the diaphragm of the sub-stage condenser is but an accident in the passage of light from the object to the eye. Its influence *as an image* in microscopic vision is nil. As an image it has no more to do with the image of microscopic vision than the image of Photo. 18 has to do with the image of Photo. 14, or than the image of a window frame has to do with the image of a distant hillside, each brought separately into view by focusing a telescope pointed through an open window.

When the cuts in a film of silver, shown in Photo's 9 and

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10, were illuminated with a small axial pencil and a card held against the front lens of the objective one could see on the card, as already described [page 338], a round spot of light flanked on each side by fainter repetitions of the round spot. On taking away the card, removing the eye-piece and looking at the back of the objective one could see a picture similar to that previously seen on the card, a bright round spot flanked on either side by diffraction repetitions of the round spot, the latter in spectrum colors. These were in this instance images of an opening in the diaphragm of the sub-stage condenser, projected by the objective in the plane *FF* of Fig. 2. The lateral images in spectrum colors were the "spectra" of the Abbe theory, corresponding in number with the faint lateral repetitions of the round spot previously seen on the card. In explanation of Fig. 1 it was noted that light of different colors, with correspondingly different wave lengths, would produce interference phenomena at slightly different points on the screen. In a similar way the pencils of diffracted light brought to a focus in the plane *FF* have the rays of one color united at points a little removed from the points at which rays of another color are united; hence the spectrum colors in the diffraction images and the name "spectra." When the sub-stage axial pencil was changed to a full cone of light from the sub-stage condenser this picture in the plane *FF* was replaced by a large circular area of light, an image of the largest opening in the diaphragm of the sub-stage condenser, which image included and obliterated every indication of the small central spot and flanking "spectra." No "spectra" are to be seen back of the objective when an object is opaque and appears bright under ordinary conditions of illumination with reflected light. *So sometimes the Abbe "spectra" are present as an accident of microscope projection and sometimes they are not.*

Why do "spectra" when present appear to be so important? This apparent importance of "spectra" may be explained by means of Fig. 2. The "spectra" in the plane *FF*, near the back of the objective, are so situated that when a slotted diaphragm placed in the plane *FF* uncovers the

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"spectra," under the conditions of Fig. 2, the same diaphragm uncovers simultaneously the emitting points *A*, *B*, *C*, *D* and *E* of the projecting lens. If now one of the slots of such a diaphragm be covered, one of the "spectra" disappears. A corresponding change occurs in the projected image. But this change is due to the loss of the slot and corresponding emitting point of the projecting lens, and not to the loss of one of the "spectra"; because, *if the "spectra" were absent, in full cone illumination, covering the slot in the diaphragm would produce the same change in the projected image.*

Let us compare Fig's 12 and 13. Fig. 12 shows paths certain primary rays travel from the source of light to the eye. Fig. 13 shows chiefly the paths certain diffracted rays of the Abbe theory travel from their origin in an object to the eye. In both diagrams let *C D* be a sub-stage condenser; *X Y Z*, an object corresponding with the object in Fig's 6, 7 and 8; *A B*, an objective; *V W*, a diaphragm having slots corresponding with [but supposed to be broader than] *4*, *1* and *5* in Fig's 6, 7 and 8; *a*, *i* and *q*, the primary ray image of microscopic vision; *F H*, a positive eye-piece; and *E*, the position of the opening in an eye-piece cap. Let *L*, *L* and *L* in Fig. 12 be light, from a somewhat distant source, which is focused at *Y* in the object by means of the sub-stage condenser. In Fig. 12 are shown above the object only such rays as leave *Y* and pass through the emitting points of the objective uncovered by slots *4*, *1* and *5* in the diaphragm *VW*. Such rays converge to a focus at *i* and then diverge towards the eye-piece, by which they are rendered nearly parallel so as to enable the eye to focus them upon the retina. In Fig. 13 *L* is a point in an opening of the diaphragm of a substage condenser, the diaphragm interrupting all but a narrow axial pencil of rays from the source of light. The primary rays leaving the point *L* are converged by the sub-stage condenser so as to be parallel. As parallel rays they pass through the object to the objective. By the objective they are focused in an image of *L* at slot *1*. They pass on each to a different point in the image *a*, *i* and *q*.

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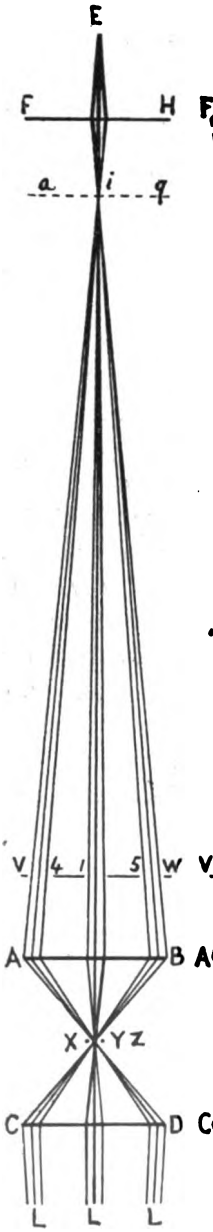

Fig. 12.

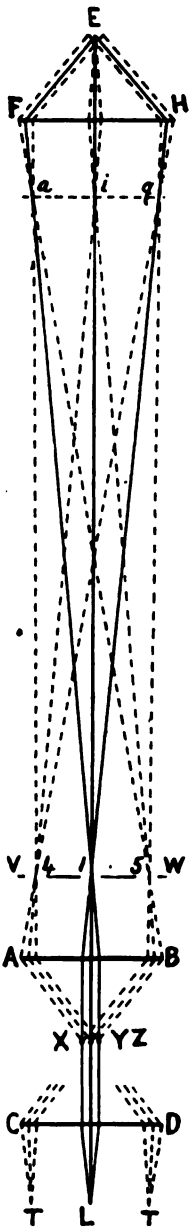

Fig. 13.

From the image *a*, *i* and *q* they pass to and through the eye-piece, by which they are focused in a second image of *L* at *E* in the opening of the eye-piece cap. From the image at *E* they diverge to such a degree as to make it *impossible for the eye to focus them upon the retina at the same time the rays shown in Fig. 12 are focused upon the retina.* The diffracted rays indicated by dotted lines have their origin in the plane of the object, and take such paths toward the objective as they would have taken if they had been primary rays starting from the points *T* and *T* in the opening in the diaphragm of the sub-stage condenser. The diffracted rays travel paths above the object similar to those just now described, and they fail to find a focus upon the retina for the same reason that the rays just now described fail to find a focus upon the retina. At slots *4* and *5* the diffracted rays form "spectra."

In Fig. 12, countless primary rays from *Y* are now supposed to pass through the slots *4*, *1* and *5* to form an

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image of *Y* at *i*. In Fig. 13, of the innumerable rays passing through the diaphragm *VW*, three only—one primary ray passing through the slot *1*, one ray diffracted by the object and passing through slot *4*, and one ray diffracted by the object and passing through slot *5*—contribute to the projection of the image of *Y* at *i*. In Fig. 12, *a* and *q* would be projected as images of *Z* and *X* as *i* the image of *Y* is projected. In Fig. 13, the projection of the images *a* and *q* is indicated as being similar to the projection of the image *i* in the same diagram. Of the rays forming the image of the point *L* at the slot *1* in Fig. 13, one ray leaves *X* and goes to the image of *X* at *q*, one ray leaves *Y* and goes to the image of *Y* at *i*, and one ray leaves *Z* and goes to the image of *Z* at *a*. We see, then, by means of the diagrams that the image of the point *L* at slot *1* in Fig. 13 is but an accident in the passage of light from the object to the image of microscopic vision, *a*, *i* and *q*. The "spectra" formed at slots *4* and *5* in Fig. 13 are also seen to be but similar accidents in the passage of diffracted light from the object to the image of microscopic vision, *a*, *i* and *q*.

In addition to the image *a*, *i* and *q* in Figs. 12 and 13, there is projected a diffraction pattern, the union of the patterns indicated in lines *I* and *II* in Fig. 9, and shown in Photo's 30 and 31, as the result of slots *4*, *1* and *5* uncovering isolated portions of aperture. Suppose now that the diaphragm *V W* in Fig. 13 be removed. The primary rays and the diffracted rays would still travel the same paths. The same isolated portions of aperture would be utilized. The image and diffraction patterns of microscopic vision would be the same. Suppose, on the other hand, that the diaphragm *VW* in Fig. 12 be removed. Such rays of the full cone *CYD* as were previously interrupted by the diaphragm *VW* would now pass on to the image of microscopic vision, *a*, *i* and *q*. Every point in the emitting surface of the objective would be effective. The diffraction pattern previously seen would not be present, except possibly as residua—such as may be seen between *a* and *i*, or *i* and *q*, in Photo. 29.

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Instead of using the word "spectra" as synonymous with images of an opening in the diaphragm of a sub-stage condenser projected in spectrum colors, let it be for a moment synonymous with rays diffracted from the object destined to reach one of the "spectra." Such rays would contribute to the projection of an image of an object just as primary rays would if the latter traveled the same paths. Both kinds of rays would form accidental images back of the objective of an opening in the diaphragm of a sub-stage condenser, or of a source of light under other but similar conditions. Neither kind of rays would have anything to do with the diffraction phenomena added to the dioptric image of an object were it not for aperture being effective in the ways we have previously studied. We have seen that the rays contributing directly to the diffraction phenomena added to a dioptric image are secondary rays originating at the emitting surface of the objective. That such secondary rays do not have their origin in the object may be shown by means of Fig. 7. At *m* is a bright point in a diffraction pattern illuminated by rays which, if they had their origin in the object, would necessarily leave—to finally reach *m* in the image—a corresponding bright spot in the object half way between *X* and *Y*, ***where there is no such bright point.***

When the axial illuminating pencil is narrow and the Abbe "spectra" are separated by well-marked intervals of darkness, the Abbe theory ignores the emitting surface of the objective corresponding with the intervals of darkness [*4–1* and *1–5* in Fig 13]. In harmony with this partial neglect of aperture, resolution in the Abbe theory may be said to increase by jumps. So long as a central image of the source of light alone is to be seen at the back of the objective [at slot *1* in Fig. 13] resolution is not present. The aperture may be increased without change in the contraction of the diffraction pattern, and in accompanying resolution, so long as the central image alone is to be seen at the back of the objective. But the moment the increase in aperture is sufficient to uncover, or admit, one flanking spectrum image [at slot *4*, or

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slot *5*, in Fig. 13] resolution is present. With greater increase in aperture no improvement in the picture as to the contraction of the diffraction pattern, and as to accompanying resolution, is to be seen until another spectrum image is uncovered, or admitted.

On the other hand, with full cone illumination, resolution increases continuously, and not by jumps or by periodic accessions. The portions of aperture neglected in the Abbe theory, those corresponding with the Abbe intervals of darkness or the portions of the emitting surface of *A B* in Fig. 12 uncovered by removing the diaphragm *V W*, are effective in full cone illumination. They contribute in proportion to their breadth, radially from the principal axis, to the contraction of diffraction patterns. And thus they may resolve additional finer details [Experiment 14, below] in an object, or increase the distinctness of the resolution of details already resolved. Periodic accessions to resolving power are only observed when the particular conditions necessary to the Abbe theory, or similar conditions, are present. Again, then, we find the Abbe theory is unsatisfactory. On the other hand, the general explanation of resolving power in optical instruments is here applicable and satisfactory.

Experiment 14: A microscope was arranged to exhibit the lines shown in Photo's 10 and 35. For the optical part of a Powell and Lealand sub-stage condenser was substituted a Powell and Lealand 1-inch objective. A Powell and Lealand 3-inch objective and a Powell and Lealand "10 compensating" eye-piece were used. A diaphragm with an opening 10 mm. in diameter was placed at the back of the objective. The revolving diaphragm of the sub-stage condenser was turned so as to bring opening "1" into use. The closer lines of the test-plate were resolved. On removing the eye-piece and looking at the back of the objective, a central image of the opening in the diaphragm of the sub-stage condenser was seen flanked on each side at the limit of the aperture by about half of an Abbe spectrum image. The more distant halves of the "spectra" were just outside the limit of the aperture and could not be seen.

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Then, for the diaphragm with an opening 10 mm. in diameter, at the back of the objective, was substituted a diaphragm having an opening 6 mm. in diameter. The latter just covered both halves of the two flanking "spectra," and left on each side of the central image a breadth of darkness corresponding with one portion of the aperture neglected in the Abbe theory. On replacing the eye-piece and again looking at the test-plate, the closer lines could not be seen. Resolution failed, because under the conditions present the Abbe theory requires for resolution the admission, by the diaphragm at the back of the objective, of at least a part of one spectrum image in addition to the central image of the opening in the diaphragm of the sub-stage condenser.

Again the eye-piece was removed. The diaphragm of the sub-stage condenser was turned so as to bring opening "3" into use. This change caused the central image seen at the back of the objective to increase in size until it filled the opening 6 mm. in diameter in the diaphragm at the back of the objective. On replacing the eye-piece and looking at the test-plate once more, the closer lines were seen. Resolution returned as a result of the additional light from the larger opening in the diaphragm of the sub-stage condenser reaching and utilizing the portions of aperture which were previously dark under the conditions necessary to the Abbe theory.

Our study of the Abbe "spectra" directs our attention to a relation existing between certain emitting points of a projecting lens, as D in Fig. 2, and the obliquity of rays diffracted by an object, as the obliquity of $a'v$ and $d'w$ in the same diagram. If the extremities of the object O, between the lines $b\,C$ and $c\,C$, were points of light in two isolated point-like holes in an opaque film of silver, the distance between them would measure a little more than a wave length of light, a little more than $r\,A$. Suppose the two holes were changed in position so that the upper one would correspond with a' and the lower one with the center of O. Let a similar hole, d', be placed as far below the center of O as a' is above the center of O. The object would then be three holes, with a distance

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between the center of O and d'' equal to that between the center of O and a'. Let parallel rays from the left be incident perpendicularly on the silver film in the plane $a'\,d''$. Primary rays passing directly through the holes towards C would be parallel to or in the principal axis of the objective. Then $a'\,v$, $O\,D$ and $d''\,w$ would be diffracted rays. The refaction of these rays by anterior lens surfaces between O and the emitting surface $A\,B\,C\,D\,E$ is not indicated. If the anterior lens surfaces were present and refraction shown, the three parallel diffracted rays, $a'\,v$, $O\,D$ and $d''\,w$, would have an obliquity of nearly 90° to the principal axis of the lens, in the case of a dry objective.

If the distance between the point-like holes subject to resolution were just equal to one wave length, the obliquity of the first effective diffracted rays leaving the object would be just 90° to the principal axis of the objective. If the distance were equal to two wave lengths, the obliquity of the first effective diffracted rays would be 30° to the principal axis; and then a second pencil of diffracted rays would be effective at an obliquity of 90° to the principal axis. If the distance were equal to three wave lengths, the obliquity of the first effective diffracted rays would be 19° 28′; and then a second pencil of diffracted rays would be effective at an obliquity of 41° 46′; and then, also, a third pencil of diffracted rays would be effective at 90°. With increasing distance between the point-like holes subject to resolution an increasing number of effective diffraction pencils of light would leave the object at obliquities of less than 90° to the principal axis of the objective, would pass through the objective, and would be emitted at points in the surface between C and D, or at points between C and B. If, on the other hand, the distance between the point-like holes were less than a wave length, and illumination were to remain axial, no effective diffracted rays would leave the object. All the diffracted rays about to leave the object would interfere one with another. Corresponding darkness would result at the front and emitting surfaces of the objective and in the

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plane *F F*. Only the point *C* of the emitting surface would be effective. Only the primary ray axial image of the opening in the diaphragm of the sub-stage condenser would be projected in the plane *F F*. The angles of obliquity* of effective diffracted rays, to the principal axis of the objective, determined by the distances between the points of detail in an object are associated with certain points in the emitting surface of an objective, such as those indicated in Fig. 2, in a way similar to that in which they are associated with the Abbe "spectra."

In our study of aperture thus far we have had in mind illumination from a source of light situated in the principal axis of the projecting lens. Only for exceptional purposes, and only by those skilled in the manipulation of the microscope and in the interpretation of diffraction phenomena, should illumination from a source of light situated in a secondary axis of the objective be used. In all ordinary work the mirror or the sub-stage condenser should be placed centrally, the center of the mirror or the axis of the condenser coinciding with the principal axis of the objective. However, under proper circumstances the resolving power of an objective may be about doubled by using sufficiently oblique illumination from a source of light in a secondary axis of the objective. Let *O B* in Fig. 2 be a primary ray from a small source of light situated in a secondary axis of the dry lens of nearly 1.00 N. A., or nearly 180° in air, passing directly through a point-like hole at the center of *O* in the film of silver. Suppose parallel incident primary rays pass through similar holes at *a'* and *d'*. Such primary rays would leave the object at an obliquity of nearly 90° to the principal axis of the objective, if the anterior lens surfaces were present and refraction shown. As the first effective diffracted rays would leave the holes at an obliquity of nearly 90° to the direct primary rays the diffracted rays would take paths in the direction *O C*. Only about half

* The English translation of THE MICROSCOPE IN THEORY AND PRACTICE, by Professors Naegeli and Schwendener, p. 230.

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the objective would be utilized. Thus we see that the obliquity of the first effective diffracted rays might have been twice as great, or double the effective aperture might have been utilized, or the holes in the film of silver might have been half as far apart.

If the holes had been half as far apart, the direct primary rays taking the same paths, the first effective diffracted rays would have had an obliquity of nearly 180° to the direct primary rays.* The direct primary rays, after passing through the objective, would have been emitted at *B*. The diffracted rays, after passing through the objective, would have been emitted at *D*. The emitting surface between *B* and *D* would have been dark, as the result of the corresponding diffracted rays interfering with one another. Thus, the points *B* and *D* would have been utilized as isolated points of emission. They would have behaved as we have seen points in an aperture uncovered by two widely separated slots behave. The resulting picture would have differed from that desired as Photo. 2, or 3, or 4, differs from Photo. 1. *Then Photo's 2, 3 and 4 are a pictorial warning for a second time, now a warning against the use of oblique illumination in ordinary work as a means of increasing or of attempting to exhaust the resolving power of the microscope. At the same time it becomes evident that every sub-stage should be provided with a means by which its condenser may be acccurately centered, and that every student using the microscope should be familiar with a method of centering his sub-stage condenser. These general rules should be accompanied by another: the sub-stage condeuser† should be as well made as the best objective, and should be used with an ever present appreciation of its power to improve or injure the picture of microscopic vision.*

It is theoretically barely possible by means of homogeneous immersion and the use of violet light to about double the resolving power of an objective, as compared with another of the same focus used dry with white light illumination.

* *Ibid.*

† Powell and Lealand "apochromatic" and "achromatic" sub-stage condensers are types of high quality. This cannot be said of the Abbe sub-stage condensers so commonly used in laboratory work. Consult the Dallinger-Carpenter edition of THE MICROSCOPE AND ITS REVELATIONS, from page 248 to page 263.

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So, while BD in Fig. 2 indicates nearly the greatest aperture [nearly 180° in air, or nearly 1.00 N. A., or nearly an effective diameter of a quarter of an inch in a $\frac{1}{8}$-inch objective] obtainable in a dry objective, AE in the same diagram indicates the greatest possible aperture theoretically obtainable with ordinary objectives by the use of homogeneous immersion and shorter wave lengths. With the use of extremely oblique light we have found that resolving power in a given objective is about double what it is with principal axis illumination. Thus, with oblique light the aperture AE should theoretically resolve points or lines about a quarter of a white wave length apart, or about 190,000 points or lines to the inch. The extreme limit of resolving power shown in the Abbe numerical aperture table is 193,037 lines to the inch. [In this paragraph the circular aperture is supposed to be the equivalent in resolving power of a corresponding rectangular aperture.]

Practically, under the conditions suitable for best microscopic vision [p. 371], the theoretical limit of resolving power found in the Abbe numerical aperture table cannot be realized. The extreme theoretical limit of the table is that which would be "about on the point of" realization with a rectangular aperture and with the use of extremely oblique illumination and violet light. But in practice we use a circular aperture and—under the conditions suitable for best microscopic vision—principal axis illumination. Moreover, Mr. Nelson* finds that objectives transmit violet light only feebly. He also finds that while the use of blue light increases the resolving power of narrow aperture objectives it gives no better results as to resolution than the use of white light in the case of a wide aperture objective. For these reasons, any example of actual resolution noted in the first column of the Abbe numerical aperture table is much less than the corresponding theoretical resolution found in another column of the table.

1.00 N. A. is the equivalent in resolving power of an aperture of 180° in air. Therefore, in harmony with our

* *Journal of the Royal Microscopical Society*, 1893, p. 15.

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study of a rectangular aperture of 180° in air, from page 388 to page 391, 1.00 N. A. should with principal axis illumination resolve points or lines one wave length apart. In harmony with our study of the same aperture with the same illumination, at page 340, points or lines half a wave length apart should "be about on the point of resolution" by 1.00 N. A. Then, in harmony with both these results, our theoretical limit of resolving power for 1.00 N. A. in the case of a rectangular aperture should be the resolution of points or lines less than one wave length apart and more than half a wave length apart, or a number of lines to the inch between 46,666 and 93,333, a wave length of white light measuring $\frac{1}{70000}$ inch. The exact number of lines an observer may see through an objective of 1.00 N. A. is uncertain. Varying keenness of vision is a factor in the uncertainty. Another factor in the uncertainty is the varying distinctness of the image of each of the points or lines subject to resolution, due to peculiarities* in the correction of the aberrations of the objective or to the use of monochromatic light for illumination.

The results actually obtained by Mr. Nelson place the limit of resolution for 1.00 N. A. with white light and principal axis illumination at about 70,000 lines to the inch. This result was obtained with a three-fourths cone of light between the object and the objective and with a circular aperture. It is interesting to note that 70,000 is three-fourths of 93,333 or that 70,000 stands just half way between 46,666 and 93,333. We may say that in practice 1.00 N. A. resolves points or lines a wave length and a half apart. By referring to Fig. 10 we see also that it may be said that a circular aperture should thoroughly resolve, in ordinary correct use of the microscope, the details of an object which would "be about on the point of resolution" by a square aperture having a diagonal equal to the diameter of the given circular aperture; for the diameter of the circle *A B C D* is a little less than the diagonal of the square bounded above by

* *Ibid*, p. 10.

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uv, below by *wx*, on the left by slot *4* and on the right by slot *5*, the breadth of the square being equal to three-fourths of the diameter of the given aperture. If the limit of actual resolution be about 70,000 lines to the inch for 1.00 N. A., the limit for 1.40 N. A., nearly the highest numerical aperture in common use, is about 98,000 points or lines to the inch.

The more important results of our experimental study of aperture may now be summarized. It appears that diffracted rays leaving an object may be considered in the same category with other rays changed in direction by an object and that the diffraction phenomena seen in a projected image are essentially the effect of changes in light above the objective due to a function of aperture, and not to changes below the objective due to diffraction of light in the plane of the object. It also appears, however, that diffraction in the plane of the object does, under some circumstances, furnish light to certain parts of an aperture from which primary rays are absent and thus enables aperture to more fully determine the character of the projected image—resulting in a more nearly truthful image or, on the other hand, in false appearances: this is the gist of the Abbe phenomena of microscopic vision. But it appears, too, that such phenomena are not peculiar to microscopic vision, notwithstanding Prof. Abbe's claim to the contrary. Moreover, with any positive lens similar and more brilliant results may be got by utilizing corresponding isolated pencils of primary rays instead of isolated pencils of diffracted rays. Still more trustworthy results may be got by using continuous apertures three-fourths [in diameter] full of primary rays instead of the isolated pencils of primary rays.

An advantage peculiar to using narrow cone illumination, the only illumination admissible in the Abbe theory with its indispensable "spectra," with an objective of wide aperture has received particular attention in this paper [for the first time, so far as the writer knows]. It has been shown that a circular aperture diaphragmed down to be

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approximately the equivalent of a narrow rectangular aperture and filled with primary rays gives the acme of trustworthy resolving power possible in the corresponding diameter of the objective. But resolving power is reduced in all other diameters. Images projected by rays passing through such an aperture would be distorted essentially as the images of the dots *a* and *b* in Fig. 3 are shown to be at *a'* and *b'*, or at *a'''* and *b'''*. It has been shown that by means of narrow cone illumination and an objective having a wide and uncovered aperture it is possible under suitable conditions to get approximately the acme of resolving power simultaneously in each of several diameters as a result of our so-called "selective power," the above distortion being incidentally eliminated. *Thus a circular aperture is approximately squared or made rectangular as to resolving power in several of its diameters simultaneously.* This approximate equivalent of "squaring the circle" as to resolving power is the advantage referred to as being peculiar to narrow cone illumination with an objective of wide aperture.

Finally, special attention is called to the fact that the Abbe theory deals with complex objects; for only such objects are subject to resolution. Single particles and uniform areas are outside its domain. These latter, however, are microscopic objects; and all objects are essentially different shaped aggregations of points. The visibility of an isolated point-like particle resembles the visibility of a star. No matter what the distance of the star, it may be seen if it has sufficient intensity. In a similar way, an isolated point-like particle—no matter what its minuteness—may be seen if it present sufficient contrast with the surrounding microscopic field. The size of the disc image in either case is no less than a limit determined finally by aperture. That limit in size, varying inversely with aperture, determines the limit of resolving power. This is the gist of the theory of microscopic vision which harmonizes with our experimental study of aperture.

It has seemed to the writer that an attempt to explain the

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projection of an image of a complex microscopic object while ignoring the projection of the images of the points of which all objects may be considered aggregations resembles an attempt to explain, for example, the functions of a complex organism while ignoring the functions of its cells. An opposite course is more natural. In harmony with this more natural course the writer has attempted to present to those who may be interested an experimental study of aperture as a factor in microscopic vision.

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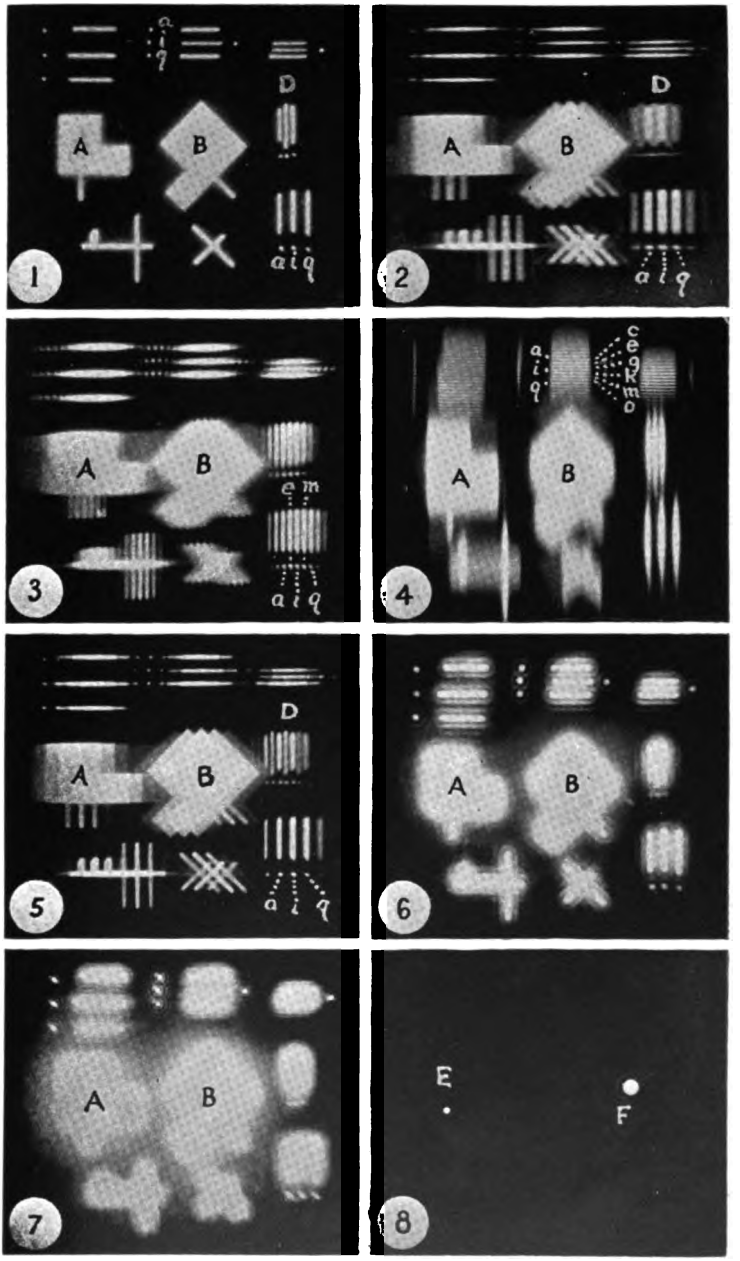

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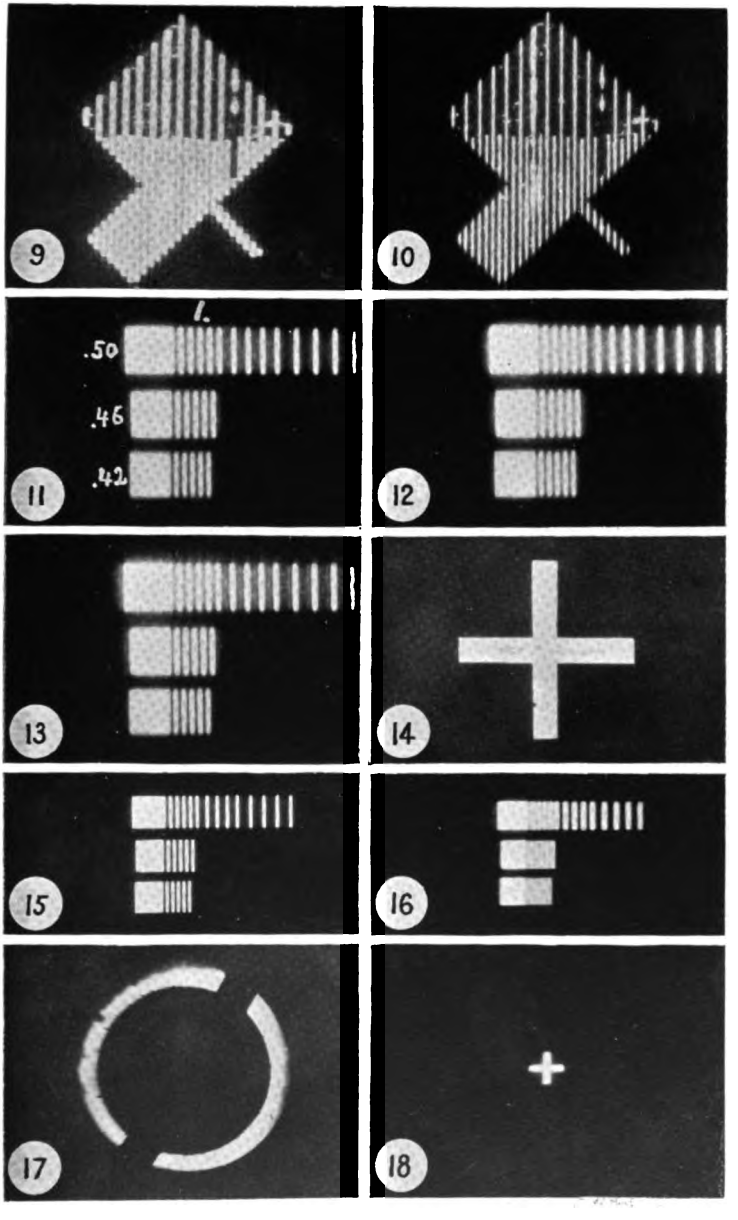

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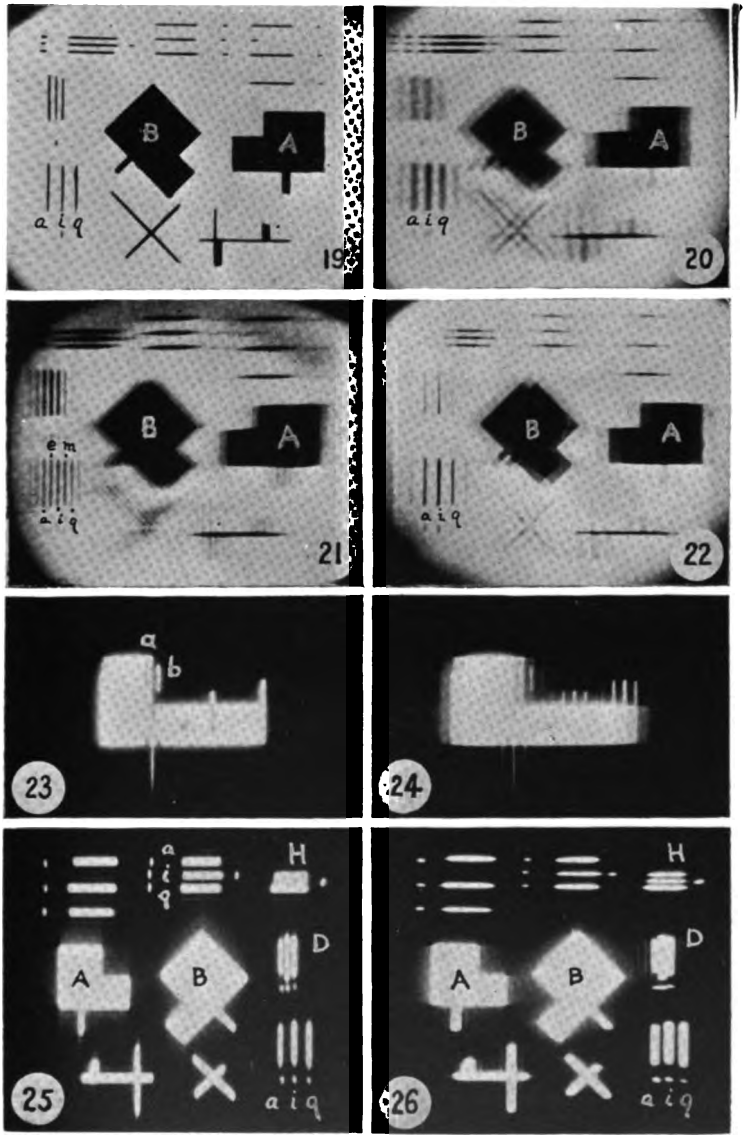

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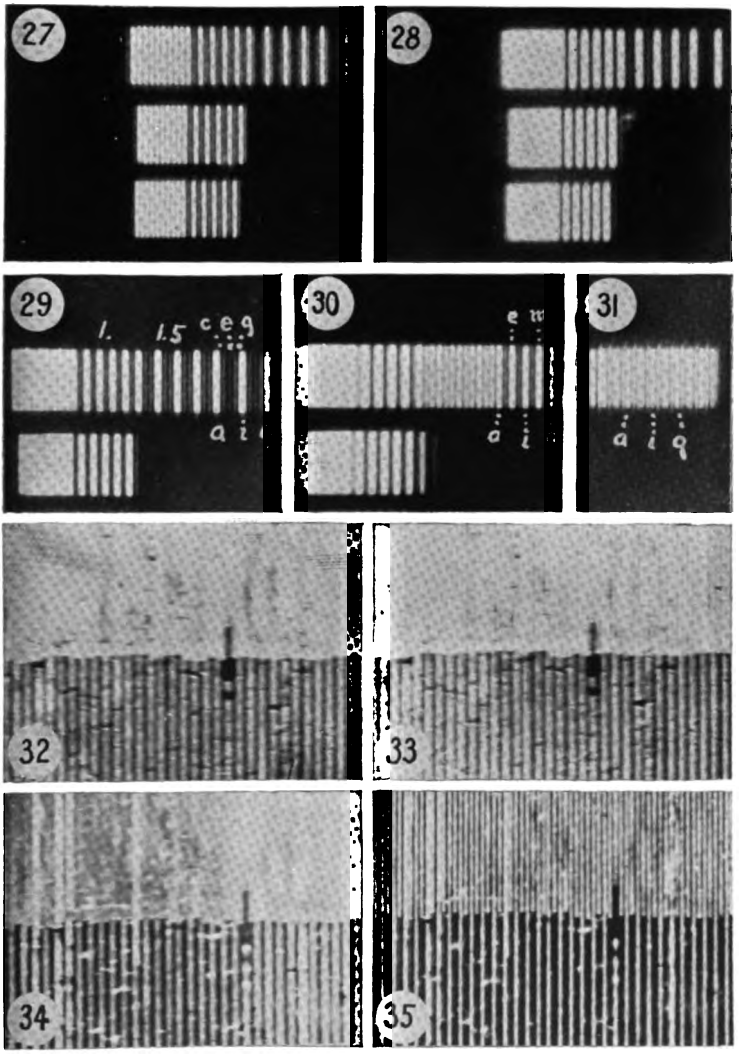

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Necrology.

JAMES E. REEVES, M. D., ... January 4, 1895.
SYLVANUS A. ELLIS, M. D., ... March 24, 1896.
GUSTAVE GUTTENBERG, PH. D., . . June 29, 1896.
LOUIS LAST, PH. G., ... August 7, 1896.

JAMES EDMUND REEVES, M. D.

Died at Chattanooga, Tenn., January 4, 1895, in his 67th year.

Dr. Reeves had been ill for a relatively short time with some obscure affection of the liver. He was born in Annisville, Va., and after an apprenticeship in the office of his preceptors, began at the age of twenty-one the practice of medicine in Sutton, W. Va. A year later he took a course of lectures at Hampden Sydney Medical College, of Richmond. Nearly ten years later he took a second course of lectures in the Medical Department of the University of Pennsylvania.

While a resident of Wheeling, W. Va., he agitated the subject of municipal hygiene and secured the passage of an ordinance establishing a city health department. In 1869, he was elected city health officer and county physician, and continued in office for four years. Subsequently he served as a member of the city council for four years. He was the author of a law creating the State Board of Health of West Virginia, of which he was a member, and for five years the secretary. For about the last six years he lived at Chattanooga.

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Dr. Reeves was one of the founders of the American Public Health Association, and its president in 1885. In 1867, he issued a call for the organisation of the State Medical Society of West Virginia, whose first secretary he was, and in 1881 its president. He was a member of the Executive Committee for West Virginia of the International Medical Congress, held at Philadelphia, in 1876. In 1882, he was elected a member of the Judicial Council, and in 1895, a trustee of the American Medical Association and a member of the Association of American Physicians. He was one of the vice-presidents of the section of Public and International Hygiene of the International Medical Congress, held at Washington in 1887; vice-president of the American Microscopical Society in 1886; a member of the Advisory Council of the Pan-American Medical Congress, held at Washington in 1893. He was employed by the State Board of Health of Tennessee to make sanitary inspections of the State's defences against yellow fever during the Jacksonville epidemic of 1888.

In addition to numerous journal articles, Dr. Reeves was the author of A Practical Treatise on Enteric Fever, and A Manual of Medical Microscopy for Students, Physicians and Surgeons. W. H. S.

PROFESSOR GUSTAVE GUTTENBERG.

Born May 10, 1843, died June 29, 1896.

By MAGNUS PFLAUM.

Science requires three classes of labor; there must be gathering by investigators, arranging and understanding by philosophers and spreading by teachers; and it would be difficult to determine which of the three achieves the greatest good for human progress and civilisation. Professor Guttenberg belonged to the last class, and by nature and training he was unusually well-fitted for his duties.

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PROFESSOR GUSTAVE GUTTENBERG.

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From what is known of his early life, it is not certain that he intended to become a teacher. After finishing his education at Vienna, Austria, with a degree of Ph. D., he became the art correspondent at Paris and London of a Vienna newspaper. He came to this country and spent almost five years traveling in the pursuit of his favorite study, mineralogy, and gathering specimens *in loco*. After two years of journalism at Wheeling, W. Va., he became a teacher of languages and science, in 1879, at Erie, Pa., and in 1889 entered upon his useful career as teacher of biology at the Pittsburg Central High School, since which time scientific studies received his sole attention. His success in teaching science was as remakable as his erudition. He had the true teacher's quality of imparting and arousing enthusiasm in his pupils, and by consummate patience, tact and judgment could smooth the roughest road and make the study of nature a pleasant task. As a result he was adored by those he instructed.

He had a kind and generous heart and was unselfish and self-sacrificing. Although one of the busiest of men, an appeal to him for aid in any scientific work, as an opinion, advice, assistance to an individual, or a paper, a lecture, or any service for a society, received the kindliest response. This promise was sacred and its performance was of a thoroughness as delightful as it was instructive to the recipients of his favors.

He was a voracious student and what he learned he remembered, and there was no limit to his studies excepting time and his physical endurance. Those who enjoyed his acquaintance were amazed and surprised at the scope and accuracy of his almost encyclopedic information, and had continual evidence of his singular love of knowledge and his familiarity with the whole range of natural science.

With a devotion to study and the most conscientious performance of duties he combined a purity of soul and nobility of character as befitted a student of nature. He was one of the High-Priests. And withal he was as gentle and modest as a maiden and cared least for public praise or approbation.

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He lived a life of duty nobly performed. His last work was the installation and opening of the museum of the Carnegie Library for the inauguration of the building, November 5, 1895. Although the means and the time given him were wholly inadequate, he fully and creditably succeeded. But the wear and tear told heavily upon his already weakened constitution. Before the final completion of the work his health gave away, and he died the following June.

His time did not permit him to render much service to this society. He attended but one, the Washington meeting. Had his life been spared he would have given more time to microscopy. Being connected with the museum as one of the trustees, and the president of the Iron City Microscopical Society, which meets in the museum lecture-rooms, he had planned a school of microscopy free to the public. Popularisation of science was his constant aim, and his early death proved a public loss.

In him passed away one of the unknown great men.

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CONSTITUTION.

ADOPTED AT ROCHESTER, N. Y., 1892.

ARTICLE I.

This Association shall be called the AMERICAN MICROSCOPICAL SOCIETY. Its object shall be the encouragement of microscopical research.

ARTICLE II.

Any person interested in microscopical science may become a member of this Society upon written application and recommendation by two members and election by the Executive Committee. Honorary members may also be elected by the Society on nomination by the Executive Committee.

ARTICLE III.

The officers of this Society shall consist of a President and two Vice-Presidents, who shall hold their office for one year, and shall be ineligible for re-election for two years after the expiration of their terms of office, together with a Secretary and Treasurer, who shall be elected for three years and be eligible for re-election.

ARTICLE IV.

The duties of the officers shall be the same as are usual in similar organisations; in addition to which it shall be the duty of the President to deliver an address during the meeting at which he presides; of the Treasurer to act as custo-

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dian of the property of the Society, and of the Secretary to edit and publish the Proceedings of the Society.

ARTICLE V.

There shall be an Executive Committee, consisting of the officers of the Society, three members elected by the Society, and the past Presidents of the Society and of the American Society of Microscopists.

ARTICLE VI.

It shall be the duty of the Executive Committee to fix the time and place of meeting and manage the general affairs of the Society.

ARTICLE VII.

The initiation fee shall be $3.00, and the dues shall be $2.00 annually, payable in advance.

ARTICLE VIII.

The election of officers shall be by ballot.

ARTICLE IX.

Amendments to the Constitution may be made by a two-thirds vote of all members present at any annual meeting, after having been proposed at the preceding annual meeting.

BY-LAWS.

I.

The Executive Committee shall, before the close of the annual meeting for which they are elected, examine the papers presented and decide upon their publication or otherwise disposal of them.

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All papers accepted for publication must be completed by the authors and placed in the hands of the Secretary by October 1st succeeding the meeting.

II.

The Secretary shall edit and publish the papers accepted, with the necessary illustrations.

III.

The number of copies of Proceedings of any meeting shall be decided at that meeting.

IV.

No applicant shall be considered a member until he has paid his dues. Any member failing to pay his dues for two consecutive years, and after two written notifications from the Treasurer, shall be dropped from the roll, with the privilege of re-instatement at any time on payment of all arrears. The Proceedings shall not be sent to any member whose dues are unpaid.

V.

The election of officers shall be held on the morning of the last day of the annual meeting. Their term of office shall commence at the close of the meeting at which they are elected, and shall continue until their successors are elected and qualified.

VI.

Candidates for office shall be nominated by a committee of five members of the Society. This committee shall be elected by a plurality vote, by ballot, after free nomination, on the second day of the annual meeting.

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VII.

All motions or resolutions relating to the business of the Society shall be referred for consideration to the Executive Committee before discussion and final action by the Society.

VIII.

Members of the Society shall have the privilege of enrolling members of their families (except men over twenty-one years of age) for any meeting on payment of one-half the annual subscription ($1.00).

Approved by the Society, August 11, 1892.

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LIST OF MEMBERS.

The figures denote the year of the member's election, except '78, which marks an original member. The TRANSACTIONS are not sent to members in arrears, and two years' arrearage forfeits membership. (See Article VII. of the Constitution.)

Members Elected at Pittsburg, Pa., 1896.

For addresses see regular list.

ANGLE, EDWARD J., B. S.
BARKER, ALBERT S.
BARTLETT, CHARLES JOSEPH, M. D.
BEAL, Prof. JAS. HARTLEY
BODINE, Prof. DONALDSON
BOYCE, JOHN W, M. D.
BRAY, THOMAS J.
BREDIN, GEO. S.
BURNER, NATHAN L.
CARPENTER, THOS. B., M. D.
CLARK, GAYLORD P., M. D.
CLARK, GEORGE EDW., M. D.
CUNNINGHAM, M. C.
DEAN, N. B. H., M. D.
DEFENDORF, ALLEN ROSS, M. D.
DIEHL, ALFRED C., M. D.
DORR, L. BRADLEY, A. B, M. D.
DORT, MISS ELIZABETH
EVANS, CHAS. H., M D.
FERRIS, Prof. HARRY B.
FLEMING, MISS MARY A.
GATES, ELMER
GREEN, MISS ISABELLA M., M. S.
HAYS, JOS. A.
HEALD, GEORGE HENRY, M. D.
HOFFMAN, JOS. H, M. D.
KELLOGG, CLIFFORD WALCOTT, M. D.
LEIPPE, J. HARRY
LOTHROP, EARL P., M. D.
MARSHALL, COLLINS, M. D.
MATSON, EUGENE G., M. D.
MEADER, LEE DOUGLAS, M. D.
MORCK, AUGUST
OLSEN, ALFRED BERTHIER, M. D.
RICE, FRANCIS SCOTT
SAMPSON, ALLEN W., M. D.
SCHMITZ, HENRY
SCHWERDTFEGER, LOUIS CHARLES, ESQ.
SMITH, CHAUNCEY P., M. D.
STEWART, ALONZO, M. D.
STOCKWELL, RODNEY R.
STONEY, ROBERT J., JR.
THOMA, FRIDOLIN, M. D.
THOMAS, MISS MARY E, Ph. B.
THORNBURY, FRANK J., M. D.
TIMMINS, GEORGE
TWINING, FREDERICK E

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